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METHODS TO SELECT CHEMICALS FOR IN SITU BIODEGRADATION OF FUEL HYDROCARBONS

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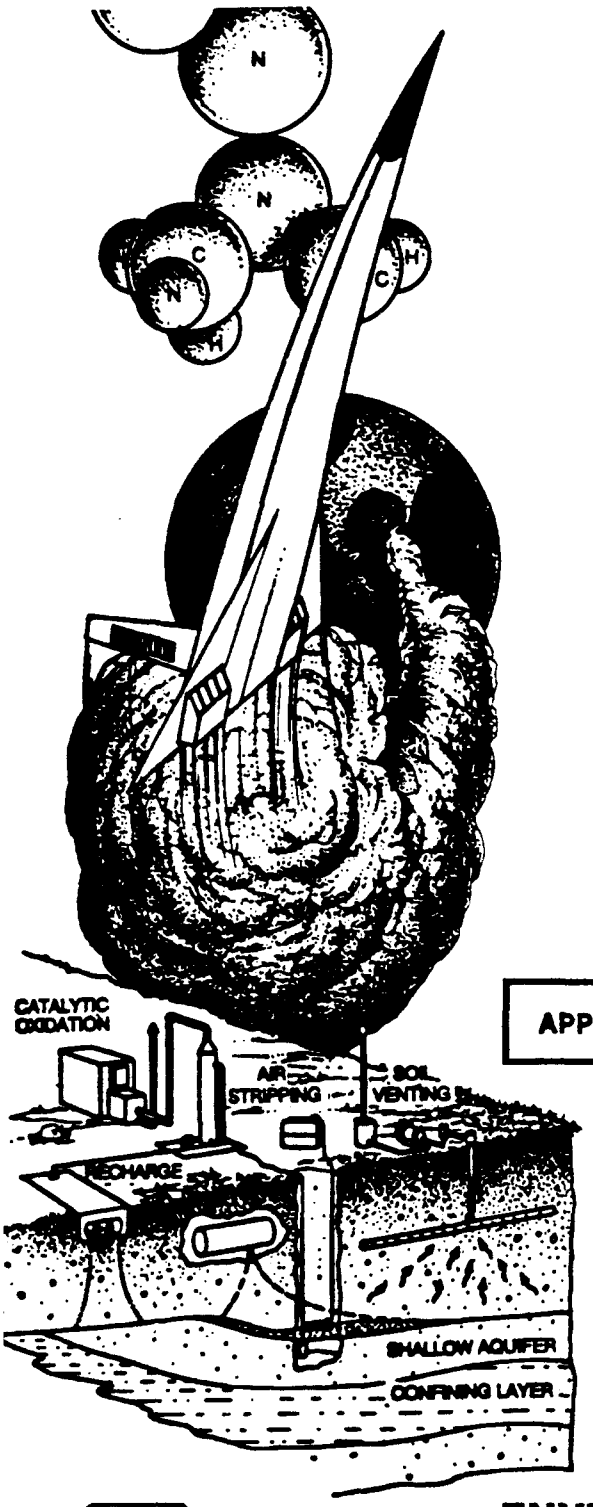

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The purpose of this effort was to improve the effectiveness of enhanced biodegradation methods to decontaminate soils and ground waters polluted by JP-4 jet fuel spills. Laboratory studies were conducted to improve nutrient formulations, reduce in situ precipitation and test potential stablilizers for hydrogen peroxide. This laboratory study has investigated the geochemical reactions which cause aquifer plugging and tested several phosphate compounds with improved solubility characteristics. Trimetaphosphate was shown to be least reactive with soils and exhibited the best groundwater transport. Several stabilizing agents for hydrogen peroxide were tested in batch and column studies. Only citrate showed promise as a stabilizing agent in batch studies, however, its performance in an aquifer simulator was unimpressive. The laboratory procedures and geochemical models used in this project can assist scientists and engineers in determining the feasiblity of site specific applications of this technology.

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SUMMARY

Nutrient formulations presently used to enhance the in situ biodegradation of fuel hydrocarbons pose two major problems: (1) plugging of the aquifer due to excessive precipitation of phosphates and (2) insufficient delivery of oxygen due to rapid decomposition of hydrogen peroxide. The objectives of this study were to develop (1) modified or new nutrient formulations to decrease plugging and the rate of peroxide decomposition and (2) a set of diagnostic procedures to select nutrient formulations for a particular site. An extensive literature review identified a large volume of information pertaining to nutrient solutions used for in situ bioreclamation and pertinent geochemical considerations. Some of the principal conclusions were as follows.

There are apparently two schools of thought on microbial nutrient requirements. One school suggests adding nutrients in excess to maximize degradation. This is the state of the practice in most current bioreclamation studies. More recently, a school has emerged that suggests that reduced nutrient levels may be adequate in some cases because it is the oxygen that is usually limiting. Concerns over precipitation/plugging problems clearly pertain to the former but are not strictly confined to situations involving excess nutrients as precipitation may also occur at lower nutrient loadings. Precipitation/plugging concerns are also present when hydrogen peroxide is used as the oxygen source, in that phosphates and polyphosphates are the most frequently used peroxide stabilizing agents.

The chemical compositions of 12 different nutrient formulations are compiled. The addition of micronutrients does not seem to be necessary, because most groundwaters probably already contain sufficient concentrations. Several inorganic and organic compounds are known to reduce the catalytic decomposition of hydrogen peroxide. Phosphate (in both the orthophosphate and polyphosphate forms) is the commonly used peroxide stabilizer in nutrient

formulations. However, phosphate apparently deactivates only the inorganic catalysts and does not react with catalase. Because catalase appears to be the most important catalyst, the need for an enzymatic inhibitor is indicated. In selecting such inhibitors, however, it must be ascertained that deactivating the catalase will not seriously impair the ability of the microbes to biodegrade hydrocarbons by increasing their susceptibility to the toxic effects of chemicals that catalase protects them from.

While chelating agents and crystal nucleation and growth inhibitors may have a role in suppressing precipitation, their presence is generally undesirable because organic chelators will exert an oxygen demand and possibly mobilize toxic metals. Polyphosphates are attractive choices relative to the organic chelators because they exert minimal oxygen demand and hydrolyze to the nutrient, orthophosphate. Chelating agent specificity for certain metals may be enhanced by geochemical equilibria modeling.

Literature on liquid fertilizers was consulted, as was the literature on solution mining. The fertilizers literature provided some valuable data on polyphosphate chemistry. Solution mining literature is highly specific to the element being mined and is not as useful.

The geochemical literature was reviewed for data on pertinent chemical reactions. A general summary of the types of information collected is provided herein. Selected nutrient formulations were modeled for improvement of chemical stability. The modeling results indicated a strong potential for the precipitation of phosphates regardless of the formulation or the composition of the groundwater. Modifications in pH or other parameters, except for a reduced phosphate concentration, may not decrease the precipitation of phosphates. The modeling results were validated by conducting long-term laboratory experiments.

In addition to geochemical modeling, a limited effort was made to model the nutrient requirements for microbial growth. These calculations indicated that oxygen probably is the limiting nutrient in most enhanced biodegradation

operation. Based on the literature search and the modeling results, it was concluded that nutrient formulations may be modified to include polyphosphates as a primary source of phosphorous and one or a combination of the several catalase inhibitors as peroxide stabilizers.

Laboratory experiments were conducted to evaluate the performance of the polyphosphates and peroxide stabilizers and to assess the nonbiological oxygen demand. Three polyphosphate species, pyrophosphate (PP), tripolyphosphate (TPP), and trimetaphosphate (TMP), were used as a nutrient phosphate source. Sandy and calcareous soils from two locations in Florida, the Eglin and Homestead AFBs, were used. In the sandy soils from Eglin, both PP and TPP were hydrolyzed rather rapidly and were sorbed on the soil. The hydrolysis of TMP was slower than the other two species. In addition, TMP did not show much sorption on the soil. These results suggested that TMP may provide a good alternative to orthophosphate.

Nutrient formulations containing any of the phosphates may not be used for biodegradation in calcareous soils, such as from the Homestead AFB, Florida. In such soils, the available Ca content and the sorption capacity of the soils are high. These properties lead to a nearly complete removal of added phosphate by sorption.

Several of the inorganic and organic peroxide stabilizers identified in the literature search were evaluated. In batch experiments with the Eglin soil, TMP and citrate were effective in decreasing peroxide decomposition by inorganic catalysts. Citrate indicated some potential for suppressing the enzymatic catalysts. In larger-scale experiments using a 6-foot long aquifer simulator, however, the rate of peroxide decomposition was not reduced even to allow transport over a soil zone of 1.5 inches corresponding to a travel time of ~2.5 hours.

The nonbiological oxygen demand of the Eglin soils was negligible and tended to decrease after a reaction time of ~24 hours. The decrease in oxygen

demand of the soil was likely due to the formation of a protective coating of oxidized minerals.

It is recommended that nutrient formulations for a particular site be selected according to procedures described in this report. These procedures are designed to provide a realistic understanding of the site-specific performance of the formulations and the extent of plugging that may occur as a result of the in situ biodegradation operation.

PREFACE

This report was prepared by Battelle Columbus, 505 King Ave, Columbus OH Zip Code 43201-2693, under Conract Number FO8635-85-C-0122, Subtask 3.05, for the Air Force Engineering and Services Laboratory (AFESC/RDVW), Tyndall AFB FL 32403-6001.

This report summarizes work done between 1 November 1988 and 30 January 1990. The HQ AFESC/RDVW project officer was Mr Douglas C. Downey.

This report has been reviewed by the Public Affairs Office amd is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nationals.

This report has been reviewed and is approved for publication.

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SECTION I

INTRODUCTION

A. OBJECTIVE

The objective of this work was to develop procedures for selecting nutrient formulations that would minimize the problems of plugging and hydrogen peroxide decomposition during enhanced, in situ biodegradation of hydrocarbon contaminated soils.

B. BACKGROUND

In situ biodegradation of fuel hydrocarbons by indigenous microbial populations is a promising technique for the remediation of contaminated soils. Enhancement of indigenous microbial activity by adding nutrients and oxygen is necessary to achieve significant biodegradation in an accelerated period. Several nutrient formulations have been used for enhancing microbial activity in situ. These solutions contain various proportions of ammonium, phosphate, and nitrate salts along with micronutrients. In most cases, the amount of phosphate in the nutrient solutions is greater than what is actually needed for normal biological growth in the subsurface. The excess phosphate has been added to attempt to decrease the decomposition rate of hydrogen peroxide, which is used as an oxygen source.

Adding chemicals in enhanced biodegradation operations frequently causes unstable geochemical environments. The two main problems associated with nutrient solution addition are (1) plugging of the subsurface formations caused by excessive precipitation, and (2) lack of sufficient oxygen caused by rapid decomposition of hydrogen peroxide.

C. SCOPE/APPROACH

The task was begun with a review of the chemical/geochemical literature to identify (a) existing nutrient formulations, (b) precipitation reactions responsible for plugging, (c) inorganic reactions exerting a nonbiological oxygen demand on the system, (d) additive compounds that may stabilize

peroxide in situ, and (e) chelating agent and crystal nucleation and growth inhibitors that may lower the extent of precipitation.

In addition, the soil biochemistry literature was reviewed to identify microbial nutrient requirements for field applications and to develop nutrient formulations based on these requirements.

The literature review was followed by modeling the geochemical consequences of using existing nutrient formulations in a variety of sites with differing groundwater compositions. The modeling effort was focused on evaluating chemical parameters (pH, combination of salts, redox, etc.) that may minimize plugging in the aquifer.

Alternative formulations were then developed based on the results of literature search, and modeling and bench-scale experiments were conducted to evaluate the performance of alternative formulations in minimizing plugging and increasing the stability of peroxide. The results of the modeling and experimental effort were then used to develop a set of procedures for selecting nutrient formulations for a particular site and for predicting the extent of plugging in the aquifer.

SECTION II

LITERATURE REVIEW

A. MICROBIAL NUTRIENT REQUIREMENTS

Biodegradation of carbonaceous substrates in soil and groundwater requires a suite of inorganic nutrients to support microbial growth, including nitrogen (N), phosphorus (P), potassium (K), sulfur (S), magnesium (Mg), calcium (Ca), and micronutrients. A typical method for determining the amount of inorganic nutrients needed to enhance biodegradation is based on the ratio of carbon (C), N, and P (C:N:P) in microbial cells compared with the C:N:P ratios in the environmental matrix, including the hydrocarbon substrate. For example, the C, N, and P content of mixed bacterial populations is on the order of 100 parts C to 10 parts N to 1 part P (Atlas, 1981). Therefore, when hydrocarbons are added to soil or groundwater, the amount of other nutrients required to balance the extra carbon demand can be calculated based on the comparative ratios of nutrients in bacterial cells. This approach assumes complete conversion of substrate to biomass. However, since only a portion of the substrate hydrocarbon is converted to biomass, and the remainder is evolved, for example as CO_2, the actual C:N:P ratios should be higher than theoretically necessary. Thus, nutrients at a variety of C:N:P ratios have been added to bioreclamation sites, often with equivocal results (Atlas, 1981; Bossert and Bartha, 1984). Adding more nutrients than required, however, may cause problems such as precipitation of salts and plugging of pores spaces.

This approach to supplying inorganic nutrients is based on the <u>total</u> hydrocarbon concentration in the environmental matrix, not on the hydrocarbon concentration actually available for biodegradation. That is, at any point in time, only a fraction of the total hydrocarbons in soil or groundwater are actually available for biodegradation. These are the portions either dissolved in the aqueous phase or at hydrocarbon-water interfaces (Atlas, 1981). Therefore, the instantaneous requirement for inorganic nutrients at the actual sites of hydrocarbon bioavailability is considerably less than the requirement calculated based on total hydrocarbon concentrations. The continual addition

of inorganic nutrients based on total hydrocarbon concentration could thus be excessive and lead to precipitation problems. As water-soluble hydrocarbons are biodegraded, additional hydrocarbons should be dissolved and become bioavailable, thus requiring additional inorganic nutrients. The problem is to balance the instantaneous nutrient requirement with instantaneous nutrient availability.

An alternative approach for determining the nutrient requirement is to consider the bioavailable hydrocarbon fraction. Under this scheme, which would suggest a lower instantaneous carbon demand and thus lower nutrient requirement compared with the total hydrocarbon concentration, it may be feasible to enhance biodegradation to an acceptable rate without the problems caused by excessive nutrients. For example, microbial degradation of bioavailable organic substrates tends to be proportional to the concentration of some limiting nutrient. Another way to express this relation is to consider the Monod equation of microbial growth:

$$\mu = (\mu_{max})(S/[K_s+S]) \qquad \text{(E-1)}$$

where μ is the growth rate, μ_{max} is the maximum growth rate, S is the substrate concentration, and K_s is the substrate concentration at 50 percent of the maximum growth rate (Stanier et al., 1986). A plot of μ versus S is a hyperbolic function that approaches a maximum value (μ_{max}). Therefore, at very high S concentrations, μ approximates μ_{max}; that is, the reaction is zero-order. If S is a limiting inorganic nutrient such as phosphorus, and hydrocarbons are in the aqueous phase, then the addition of minimal quantities of phosphorus should increase the growth rate in a first-order fashion until the concentration of phosphorus is increased to the point of stimulating maximum (zero-order) growth. However, as described above, the quantity of phosphorus necessary to stimulate maximum growth may be excessive and result in precipitation and plugging problems. Therefore, it may be reasonable to accept an overall lower rate of soluble carbon degradation by adding minimal levels of limiting nutrients, as opposed to aiming for a maximum degradation rate by adding excessive nutrients that may precipitate. Empirical results

(Brubaker, 1989) suggest that phosphorus and nitrogen at concentrations in the low milligrams-per-liter range are sufficient to stimulate hydrocarbon biodegradation in groundwaters supplemented with adequate oxygen.

B. EXISTING NUTRIENT FORMULATIONS

A large body of literature exists that describes nutrient formulations. However, much of this information has been derived under controlled laboratory conditions. The emphasis of this review was placed on data from field studies, although pertinent information on laboratory studies was included. Table 1 is a compilation of nutrient formulations that were described in the literature. This is not an exhaustive list but is representative of the types of nutrient formulations used. In Table 2 the formulations have been converted to milligrams/liter (mg/L) concentrations of the major constituents.

All existing nutrient formulations consist of a nitrogen source and a phosphorus source. The nitrogen sources include ammonium and nitrate salts with no obvious preference for nitrogen in the nitrate versus ammonium form. The phosphorus sources typically are orthophosphate salts of sodium, ammonium, or potassium. Most formulations also include approximately equal molar concentrations of mono- and di-hydrogenated forms, which buffer the solution at approximately pH 7. Polymeric phosphates (such as $P_3O_{10}^{5-}$) appear to be used primarily to stabilize hydrogen peroxide.

Micronutrients, for example Mn, Fe, Zn, Cu, are not present in all formulations. One of the reasons for the absence of micronutrients in nutrient formulations may be the presence of these constituents in local groundwater used for preparing nutrient solutions in the field. Although micronutrients are necessary for microbial growth, some studies have indicated that addition of micronutrients may not affect or may even lower the rate of substrate mineralization (Dibble and Bartha, 1979; Swindoll et al., 1988).

C. PEROXIDE DECOMPOSITION

Hydrogen peroxide decomposes to release oxygen and water:

$$H_2O_2 \rightarrow H_2O + \tfrac{1}{2}O_2 \qquad \text{(R-1)}$$

Many substances commonly present in groundwater and soils act as catalysts for Reaction (R-1). Important among these are aqueous species of iron and copper

TABLE 1. FORMULATION FOR NUTRIENT SOLUTIONS USED FOR FIELD OR LABORATORY BIORECLAMATION.

Component	Nutrient Concentrations, mM											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
NH_4Cl	93.5											
NH_4NO_3		1.25				6.25		12.5		0.63		1.25
$(NH_4)_2SO_4$			1088		414		7.58					
KNO_3				2.50								
$Ca(NO_3)_2 \cdot 4H_2O$				2.50								
$NaNO_3$											47.1	
Na_2HPO_4	12.8		281		106				8.45		4.20	
NaH_2PO_4	3.49		268		101							
$Na_6P_6O_{18}$	4.76											
$Na_2HPO_4 \cdot 7H_2O$		0.15										
KH_2PO_4		0.83		0.50		2.94	3.00	7.35	3.28		11.0	1.47
K_2HPO_4						9.20	3.80	11.5		2.45		4.60
$MgSO_4 \cdot 7H_2O$		0.08	7.08	1.00		0.81			0.41		0.041	
Na_2CO_3		0.95	4.21									
$CaCl_2 \cdot 2H_2O$		0.01	0.39			0.17					0.068	0.42
$MnSO_4$		0.01										
$FeSO_4 \cdot 7H_2O$		0.002	0.08						0.005		0.004	
$MnSO_4 \cdot H_2O$			0.24						0.006			
H_3BO_3				0.03								
$ZnCl_2$				0.002					0.002			
$CuCl_2 \cdot 2H_2O$				$2x10^{-4}$					0.002			
MoO_3				$2x10^{-4}$								
$MnCl_2 \cdot 4H_2O$				0.001			0.01					
$FeCl_3 \cdot 6H_2O$						0.01						
$MgCl_2 \cdot 6H_2O$							0.50					
$FeCl_2 \cdot 4H_2O$							0.26					

TABLE 1. FORMULATION FOR NUTRIENT SOLUTIONS USED FOR FIELD OR LABORATORY BIORECLAMATION (CONCLUDED).

Component	Nutrient Concentrations, mM											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
NaCl							1.87					
$MgSO_4$								1.67		0.42		0.83
$CaCl_2$								0.18				
$FeCl_3$								0.03				0.06
$CaSO_4 \cdot 2H_2O$									0.29			
$(NH_4)_6Mo_7O_{24} \cdot 4H_2O$									$2x10^{-4}$			
$Na_2B_4O_7 \cdot 10H_2O$									$7x10^{-4}$			
$Co(NO_3)_2 \cdot 6H_2O$									$9x10^{-4}$			
NH_4VO_3									$9x10^{-4}$			

References

I - Restore 375 manufactured by FMC chemicals
II - Jhaveri and Mazzacca, 1983
III - Raymond et al., 1978
IV - Hoagland's Solution (Morholt et al., 1958)
V - Raymond et al., 1975
VI - Flathman and Githens, 1985
VII - Liu and Wong, 1975
VIII - Olive et al., 1975
IX - Zeyer and Kearney, 1982
X - Kim and Maier, 1986
XI - Swindoll et al., 1988a,b
XII - Goldstein et al., 1985

TABLE 2. COMPOSITION OF NUTRIENT SOLUTIONS USED FOR FIELD OR LABORATORY BIORECLAMATION.

Component	Nutrient Concentrations, mg/L											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
NH_4^+	1,683	23	19,584	--	7,452	113	273	225	0.04	11	--	23
K^+	--	32	--	117	--	832	413	1,184	128	191	429	416
Ca^{2+}	--	0.4	16	100	--	7	--	7	12	--	3	17
Na^+	1,216	51	19,284	--	7,199	--	43	--	389	--	1,277	--
Cl^-	3,319	0.7	28	0.2	--	13	121	16	0.3	--	5	36
NO_3^-	--	78	--	465	--	388	--	775	0.1	39	2,920	78
SO_4^{2-}	--	9	105,158	96	39,744	78	728		160	78	40	480
PO_4^{3-}	2,904	93	52,155	48	19,665	1,153	646	19	1,114	233	1,444	577
CO_3^{2-}	--	57	253	--	--	--	--	--	0.2	--	--	--
BO_3^{3-}	--	--	--	2	--	--	--	--	0.09	--	--	--
VO_3^{3-}	--	--	--	--	--	--	--	--	--	--	--	--
Mg^{2+}	--	2	170	24	--	19	12	40	10	10	1	20
Mn^{2+}	0.6	13	0.06	--	--	0.6	--	--	0.3	--	--	--
Fe^{2+}	--	0.1	4.5	--	--	0.6	15	2	0.3	--	0.2	3
Zn^{2+}	--	--	--	0.1	--	--	--	--	0.1	--	--	--
Cu^{2+}	--	--	0.01	--	--	--	--	--	0.1	--	--	--

TABLE 2. COMPOSITION OF NUTRIENT SOLUTIONS USED FOR FIELD OR LABORATORY BIORECLAMATION (CONCLUDED).

Component	Nutrient Concentrations, mg/L											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
Co^{2+}	--	--	--	--	--	--	--	--	0.05	--	--	--
Mo^{2+}	--	--	--	0.02	--	--	--	--	0.12	--	--	--

References

I - Restore 375 manufactured by FMC chemicals
II - Jhaveri and Mazzacca, 1983
III - Raymond et al., 1978
IV - Hoagland's Solution (Morholt et al., 1958)
V - Raymond et al., 1975
VI - Flathman and Githens, 1985
VII - Liu and Wong, 1975
VIII - Olive et al., 1975
IX - Zeyer and Kearney, 1982
X - Kim and Maier, 1986
XI - Swindoll et al., 1988
XII - Goldstein et al., 1985

and the enzyme catalase (Schumb et al., 1955). Several mechanisms for the action of catalysts on peroxide decomposition have been described. The proposed mechanism for the reaction catalyzed by ferric and ferrous iron is as follows (George, 1952; Brown et al., 1970; Walling and Weil, 1974):

$$Fe^{2+} + H_2O_2 = Fe^{3+} + HO\cdot + HO^- \quad (R\text{-}2)$$

$$Fe^{3+} + H_2O_2 = Fe^{2+} + HO\cdot_2 + H^+ \quad (R\text{-}3)$$

$$HO\cdot + H_2O_2 = H_2O + HO\cdot_2 \quad (R\text{-}4)$$

$$Fe^{3+} + HO\cdot_2 = Fe^{2+} + H^+ + O\cdot_2 \quad (R\text{-}5)$$

$$Fe^{2+} + HO\cdot_2 = Fe^{3+} + HO_2^- \quad (R\text{-}6)$$

$$Fe^{2+} + HO\cdot = Fe^{3+} + HO^- \quad (R\text{-}7)$$

where Fe^{2+} or Fe^{3+} may be the single ions or complex ionic species of iron. The mechanism of catalytic action of copper (Cu) is similar to that of iron (Fe). In addition to the homogeneous reactions above, decomposition of hydrogen peroxide can also be catalyzed by heterogeneous reactions. These reactions involve catalytic action at the surface of iron hydroxide or oxide precipitates (Schumb et al., 1955).

Although inorganic catalysts contribute to the decomposition of peroxide in situ, enzymatic catalysts, peroxidase and catalase, may be dominant in soil - groundwater systems. Peroxidases are a group of catalysts that oxidize a substrate in the presence of hydrogen peroxide. Peroxidase activities are greatest in soils containing carbonate and may be a function of the number of soil microorganisms (Skujins, 1967). The activity of peroxidase changes with the type of vegetation and with the seasons.

Catalases are enzymes that degrade hydrogen peroxide to produce water and molecular oxygen. Their activity in soils is associated with high organic matter content. The highest catalase activity is found in litter-accumulating surface layers and in humus-accumulating horizons of the soil column. It is also found that catalase activity is stronger in alkaline and calcareous soils than in acid soils (Skujins, 1967). The catalytic action of catalase in decomposing peroxide is by far the strongest of all the inorganic and enzymatic catalysts (Table 3).

TABLE 3. CATALATIC ACTIVITY OF SEVERAL FERRIC-CENTERED CATALYSTS IN THE DECOMPOSITION OF HYDROGEN PEROXIDE (from Nicholls and Schonbaum, 1963).

Catalyst	Activity (turnover number)*
Catalase	9×10^4 (pH 7, 20°C, 0.01 mole peroxide)
Peroxidase	4.0 (pH 7, 20°C, 0.01 mole peroxide)
Fe(III)-TETA	22.0 (pH 7, 25°C, 0.15 mole peroxide)
Fe(II) or Fe(III) ion	1.0 (pH 5, 0°C)

* Turnover number = number of peroxide molecules decomposed per second by each mole of the catalyst.

Catalases are high-spin ferric complexes containing hemin as the prosthetic group; each molecule usually contains four hemin groups. Because catalase is an iron-containing enzyme, its catalytic action in peroxide decomposition is similar to that of inorganic ferric salts. Experimental studies of hydrogen peroxide decomposition by catalase indicate that there is an initial period of rapid decomposition, followed by a slower, steady rate of decomposition. The kinetics of the reaction during the steady rate of decomposition are described by the following equation (George, 1952; Brown et al., 1970):

$$-d(H_2O_2)/dt = k[\text{catalase}]\ [H_2O_2] \qquad \text{(E-2)}$$

Both the initial and steady rates of decomposition are proportional to catalase activity; variation with peroxide concentration is complex, rising to a maximum at 0.4 moles/liter and 0.07 moles/liter H_2O_2, respectively, and then decreasing rapidly (George, 1952). This self-inhibitory effect of hydrogen peroxide is not caused by the destruction of catalase; rather, it is considered to be a result of reversible formation of intermediate, enzyme-substrate compounds (George, 1952; Nicholls and Schonbaum, 1963; Brown et al., 1970).

In other words, the loss of catalase activity at high peroxide concentrations will be recovered with a decrease in peroxide concentration.

Brown et al. (1970), considering available experimental evidence, suggested the following scheme for the catalase-hydrogen peroxide reaction:

$$E + P \rightarrow EP \quad \text{(R-8)}$$

$$EP \rightarrow EP' \quad \text{(R-9)}$$

$$EP' + P \rightarrow E + \text{products} \quad \text{(R-10)}$$

$$EP' + P \rightarrow \text{Catalase Compound II} \quad \text{(R-11)}$$

where E is the enzyme, P is peroxide, and EP and EP' are intermediate compounds. Catalase Compound II has been identified as an inactive species formed in the reaction and may reversibly react with peroxide to form another inactive species, Compound III. This reversible formation of compounds II and III accounts for self-inhibition at high peroxide concentrations (Brown et al., 1970).

D. PEROXIDE STABILIZATION

The stability of hydrogen peroxide can be increased by decreasing the availability of or by deactivating the catalysts. Stannate, phosphate, and fluoride deactivate the inorganic catalysts, whereas borate, sulfide, hypophosphite, fluoride, and several organic compounds reduce the catalytic action of catalase.

1. Inhibition of Inorganic Catalysts

The catalytic action of Fe is decreased by adding stannate or phosphate to hydrogen peroxide solutions (Schumb et al., 1955). Adding sodium stannate forms colloidal hydrous stannic oxide, which adsorbs catalytic ions such as Fe^{3+} and improves the stability of hydrogen peroxide.

The role of the phosphate ion is to scavenge the hydroxyl radical HO· produced from Reaction R-3 and, thus, stop the chain decomposition reactions. At environmental pH ranges the species $H_2PO_4^-$ and HPO_4^{2-} predominate. Both phosphate species can act as HO· scavengers. The rate constant of HO· production is $k_1 = 76.5\ M^{-1}\ sec^{-1}$ (Walling and Weil, 1974) and for HO· scavenging is $k_7 = 5 \times 10^6\ M^{-1}\ sec^{-1}$ (Black and Hayon, 1970), indicating scavenging of the

free radicals by phosphates controls the overall rate of peroxide stabilization.

Phosphate also impedes peroxide decomposition by lowering the dissolved Fe(II) and Fe(III) concentrations by (1) precipitating Fe as phosphate salts or (2) by complexation as Fe-PO_4 ions. In addition to orthophosphate, various polyphosphates also act as peroxide stabilizers. For example, sodium pyrophosphate appears to stabilize hydrogen peroxide by either precipitating or sequestering the ionic Fe species and has been found to be an effective stabilizer in the presence of up to 10 parts per million (ppm) Fe (Schumb et al., 1955).

Moderate amounts of fluoride ions also inhibit the Fe-catalyzed decomposition of hydrogen peroxide. Thus, it appears that any chemical that reduces the soluble Fe content should have an effect on peroxide stabilization. However, not all ligands that form stable complexes with Fe are effective stabilizers. For example, while phosphate and fluoride complexes of Fe are catalytically inactive, Fe-EDTA or Fe-TETA complexes are stronger catalysts than even Fe^{3+} (see Table 3).

2. Inhibition of Enzymatic Catalysts

Stabilization of hydrogen peroxide in the presence of enzymatic catalysts, primarily catalase, is much more complex. Experimental studies of the hydrogen peroxide - catalase system have identified several inorganic and organic species that have an inhibitory effect on catalase activity. The inhibitory effect of these species arises from a reversible or irreversible formation of complexes between the inhibitors and the free enzyme or enzyme - peroxide compounds.

Inorganic inhibitors of catalase activity (Beers and Sizer, 1954; Nicholls, 1961) include fluoride, borate, sulfide, and hypophosphite (H_3PO_2). Organic inhibitors include acetate, citrate, pyrogallol, hydroquinone, catechol, hydroxylamine and several other compounds (Alyea and Pace, 1933; Schumb et al., 1955). Of these, only citrate is known to have been used previously in nutrient formulations for in situ bioreclamation. Citrate's stated purpose is to reduce the iron precipitation potential, although it may also have some

impact on hydrogen peroxide stability. The anionic inhibitors of catalase activity mentioned above can be divided into two categories (Nicholls, 1961a): (1) ligands forming ionic complexes; and (2) ligands forming covalent complexes.

Ascorbate also is known to inhibit the activity of catalase (Davison et al., 1986). Ascorbate inhibition of catalase is reversible but may be enhanced and made irreversible in the presence of oxygen or organic complexes of copper. However, the presence of both oxygen and copper apparently decreases the irreversible inhibition of catalase by ascorbate.

Fluoride and acetate form ionic complexes with catalase and catalase Compound II (see Reactions R-10 through R-13 above). In addition, fluoride and acetate both accelerate the decomposition of Catalase Compound II to free enzyme. This negates to some extent the complexes' inhibitory action. The dissociation constants of fluoride - catalase complexes are lower than those for acetate complexes (Nicholls, 1961a), indicating that fluoride should be a more effective inhibitor than acetate.

Hypophosphite also forms ionic complexes, but its reaction with catalase is slow. However, the inhibitory action of hypophosphite may be much stronger (less reversible) than that of fluoride or acetate. The following scheme illustrates this difference (Nicholls, 1961a).

$$\text{Catalase Compound II} + \text{HA} \underset{k3}{\overset{k2}{\rightleftharpoons}} \text{Catalase Compound II.HA} \qquad \text{(R-12)}$$

$$\text{Catalase Compound II.HA} \overset{k4}{\rightarrow} \text{Catalase.HA} \qquad \text{(R-13)}$$

where HA is the anionic species. For fluoride and acetate, k3 >> k4, but for hypophosphite, k3 << k4. In other words, hypophosphite accelerates the decomposition of Catalase Compound II with a concomitant formation of hypophosphite-catalase.

Sulfide forms covalent complexes with catalase and is a much stronger inhibitor of catalase activity compared with fluoride and acetate (Beers and

Sizer, 1954; Nicholls, 1961; Hewitt and Nicholas, 1963). The reactions of catalase and sulfide are summarized as follows (Nicholls, 1961b):

$$\text{Catalase Compound II} + H_2S + Fe^{2+} \rightarrow \text{Catalase.S.}Fe^{2+} + H_2O \qquad \text{(R-14)}$$

$$\text{Catalase.S.}Fe^{2+} \underset{}{\overset{O_2}{\rightleftharpoons}} \text{Catalase.S.}Fe^{3+} \qquad \text{(R-15)}$$

$$\text{Catalase.S.}Fe^{3+} \xrightarrow{O_2 \text{ (or peroxide)}} \text{Catalase.}Fe^{3+} + \text{oxidized S} \qquad \text{(R-16)}$$

Reaction (R-16) indicates the reversible nature of the sulfcatalase complex in the presence of oxygen or hydrogen peroxide. The dissociation of the complex in Reaction (R-15), ferrous sulfcatalase, to form free catalase is catalyzed by fluoride. The ferric iron in sulfcatalase also reacts reversibly with hydrogen sulfide to give sulfide-sulfcatalase. Although the sulfide-catalase compounds are reversible, they may be useful inhibitors for biodegradation process because (1) they increase the number of steps required to decompose hydrogen peroxide, and therefore, produce some stabilizing action, and (2) the regeneration of free catalase from sulfide-catalase complexes involves some amount of catalase destruction. However, it may be difficult to achieve a significant inhibitory effect using sulfide in environmental systems because of its tendency to form insoluble precipitates with metals such as Fe. Also, too much sulfide may exert an oxygen demand on the system.

The inhibitory effect of borate on catalase activity has not been studied directly. Jones and Suggett (1968) observed that the activity of catalase decreased in aqueous solutions containing the borax - sodium carbonate buffer. In a more recent study, Kelly and King (1981) also suggest inhibitory action of borate on catalase based on their experiments in a system containing iron(III)-porphyrin complexes and hydrogen peroxide.

An additional mechanism of the stabilizing action of borate involves the formation of aqueous perborate (H_4BO_5) complexes. Thermodynamic studies of the boric acid - hydrogen peroxide system (Pizer and Tihal, 1987) indicate that perborate species are dominant in the pH range of 7.5 to 13. Boric acid is dominant at pH < 7.5 and the borate ion at pH > 13. Thus, it is possible that perborates may provide an alternative source of oxygen in the pH range of

their stability if direct catalase - perborate interactions are not significant. A similar use of perborates has been suggested by Richards et al. (1988). The use of borate and possibly fluoride in bioreclamation processes, however, may be limited because excessive amounts of these constituents are toxic to plants and possibly to microbes. In addition, the use of these ions may pose questions about water quality or drinking water standards.

The organic stabilizers may be less desirable for use in bioreclamation because, if used in sufficiently high concentrations, they may exert a nonbiological oxygen demand and reduce the availability of oxygen for microbial growth. However, if a highly selective inhibitor can be identified, then only a small concentration of the inhibitor will be needed to produce significant peroxide stabilization. For example, Alyea and Pace (1933) indicate that pyrogallol, hydroquinone, catechol, and resorcinol all possess strong (and presumably selective) inhibitory powers for catalase. This aspect will be explored further in the experimental phase.

In summary, several inorganic and organic compounds are known to reduce the catalytic decomposition of hydrogen peroxide. Phosphate (in both the orthophosphate and polyphosphate forms) is a commonly used peroxide stabilizer in nutrient formulations. However, phosphate apparently deactivates only the inorganic catalysts and does not react with catalase. Because catalase appears to be the most important catalyst (see Table 3), an enzymatic inhibitor is needed. In selecting such inhibitors, however, it must be ascertained that deactivating the catalase will not seriously impair the ability of the microbes to biodegrade hydrocarbons by increasing their susceptibility to the toxic effects of chemicals that the catalase protects them from.

E. CRYSTAL NUCLEATION INHIBITION AND CHELATING AGENTS

Crystal nucleation and growth inhibitors and chelating agents are potentially useful in bioreclamation for inhibiting the precipitation of phosphate salts that could lead to plugging. There are at least two major mechanisms for inhibiting crystal nucleation and growth: (1) in the first, the inhibitor compound adsorbs to the surface of the seed crystal and physi-

cally impede crystal growth; (2) the second deals with the reduction of ion activities via chelation, making the solution undersaturated with respect to the precipitating phase. Chelating agents are also potentially useful for chemically pretreating a site. The most widely known of the chelating agents are EDTA and NTA, but others are also available.

One of the most studied families of crystal nucleation and growth inhibitors is the phosphonates (King, 1969; Miller et al., 1982; Austin et al., 1975). As a whole, the phosphonates were found to be effective calcium salt precipitation inhibitors, working in less than stoichiometric doses. Amino-phosphonic acids were found to be the most effective, with the ability to prevent deposition for extended periods of time at the high temperatures found in evaporators. Monophosphonic acids were also found to be effective under the same conditions.

Polyacrylic acids and sulfonated styrene copolymers were found to be the most effective precipitation inhibitors when compared with EDTA, NTA, NTP, and HEDP (Greenlimb and Carter, 1980). This evaluation was based on their ability to inhibit precipitation when under-, over-, or properly dosed. Of the two, sulfonated styrene copolymers were more effective than polyacrylic acids and showed little loss of effectiveness in the presence of iron.

EDTA, NTA, HEDTA, CDTA, and DTPA were found to be effective for chelating hematite (α-Fe_2O_3) (Chang et al., 1983). Other compounds that were found to be effective for chelating iron were ferrozine (Thompsen and Mottola, 1984), desferrioxamine B (Birus et al., 1984), and hydroxamic acids (Brink and Crumbliss, 1984). However, these compounds were most effective under acidic conditions. Oxo and thio ligands or hydroxamic acids were studied by Fish and Crumbliss (1985) with thiohydroxamic acid found to form a more stable chelate at neutral pH. Hydroxamic acid was also studied by Winston et al. (1985), who found it to be extremely effective for treating victims of iron poisoning and ß-thalessimia patients. Cavasino et al. (1984) studied the chelating effects of diglycolic, tartaric, and citric acids under extremely acidic conditions (pH < 1.5) and found each to complex iron(III) under these conditions.

Naturally occurring polycarboxylic acids (citric, oxalic) and 3-hydroxy-4-pyronates and polyphenols (maltol, kojic acid, gallic acid) are good

chelators for aluminum (Ohlman and Sjoberg, 1988; Lopez-Quintela et al., 1984). The chelating action of these ligands is most pronounced in the pH range of 3 to 7.

Most chelating agents show little specificity for specific elements. Competing reactions reduce the effectiveness of the chelating agent for the cation of interest and greatly increase the quantity of chelating agent that must be added to achieve the desired result. Too much chelating agent in an in situ bioreclamation project is undesirable because of (1) the oxygen demand exerted by the chelating agent, if it is biodegradable; (2) increased cost of adding more chelating agent; and (3) if nonbiodegradable, the chelating agent may increase the mobility of certain toxic metals present in groundwater. Chelating agent specificity for a certain metal may be enhanced, however, by using the appropriate chelating agent and selecting the appropriate chemical environment. Agrawal et al. (1986) chemically modeled the selective chelation of calcium phosphate salts in the presence of large amounts of competing copper and iron. From conditional stability constant theory, for example, it was shown that EGTA, a chelating agent similar in structure to EDTA, would form relatively selective complexes with calcium under mildly alkaline and reducing conditions. Also, the selectivity of EGTA could be further enhanced by adding of a chemical masking agent, in this case tetren, to complex with and reduce competition from the heavy metals. Chaney (1988) described the relatively selective chelation of iron(III) by the chelating agent, HBED, and its applicability to microelement speciation in nutrient formulations such as Hoagland's solution. It is likely that this selectivity could be even further enhanced by chemical equilibria modeling, such as performed by Agrawal et al. (1986).

Polyphosphates such as pyrophosphate are also potentially important chelating agents for use in in situ bioreclamation. They are particularly attractive because they will exert a minimal oxygen demand and readily hydrolyze to orthophosphate, which is an important nutrient. Polyphosphates are discussed further in the Geochemical Equilibria section that follows.

F. GEOCHEMICAL EQUILIBRIA

Geochemical processes of soil-solution interaction include both homogeneous and heterogeneous reactions. Homogeneous reactions, that is, equilibria in the solution phase only, are those that describe chemical speciation of various cations and anions, including oxidation and reduction. Heterogenous reactions take place between the soil and the solution phase, such as adsorption, ion-exchange, and dissolution and/or precipitation of solids.

There are two prerequisites for modeling geochemical equilibria attending biodegradation of hydrocarbons in soils: (1) computer programs for simulating important processes of soil-solution interaction; and (2) thermodynamic, kinetic, and field data that can be used to describe fully the consequences of these processes.

Several computer programs can be used to simulate both homogeneous and heterogeneous equilibria. We have acquired and installed on our computer systems three of the most commonly used codes:

- MINTEQA2 (EPA, 1988)
- SOLMINEQ88 (Kharaka et al., 1988)
- EQ3NR/EQ6 (Wolery, 1983).

Simulation of homogeneous equilibria is essentially similar in all the three codes; major differences occur in the data bases. MINTEQA2 and SOLMINEQ88 have limited capabilities for simulating the precipitation and dissolution of minerals; however, these two codes allow modeling of adsorption/ion-exchange. The EQ3NR/EQ6 code has an extensive capability to model the precipitation/dissolution process in both equilibrium and kinetic modes. A brief discussion of the application of the codes is presented here. Detailed accounts of the theory can be found in the references cited above.

Let us assume that we are to inject a nutrient solution, such as RE$_3$TORE™ 375, containing ammonium chloride and ortho- and polyphosphates of sodium into a soil where the groundwater contains iron, calcium, sodium, potassium, bicarbonate, chloride, and sulfate. Mixing the two solutions will produce changes in the chemical form of the dissolved material and may result in

precipitation. For example, calcium may exist as a single ion (Ca^{2+}) or as an ion-pair with phosphate ($CaPO_4^-$; $CaHPO_4^-$; $CaH_2PO_4^+$), sulfate ($CaSO_4^o$), carbonate ($CaCO_3^o$; $CaHCO_3^+$), and chloride ($CaCl_2^o$). Similarly, other cations present in the solution may also associate with the phosphate ion. Thus, the total (analytical) concentration of calcium and phosphorus will be given by the following:

Ca_{total} = concentration of Ca^{2+} + other species of Ca

P_{total} = concentration of PO_4^{3-} + other phosphate species

If an element can occur in multivalent states, such as iron, then speciation calculations of such elements include the distribution of oxidized and reduced species. The speciation distribution calculations will finally provide the fraction and concentration of each element that occurs as a single ion (e.g., Ca^{2+}. PO_4^{3-} etc.).

The concentrations of single ions are most important because they determine whether a mineral may or may not precipitate. For example, calcium may precipitate with phosphate if the product of the ionic activities of Ca^{2+} and PO_4^3 exceeds the thermodynamic solubility constant of any of the calcium phosphate solid phases.

The concentrations of associated species of each element also provide important information on the redox, chelation, and precipitation behavior of the soil-solution system. For example, Millero (1985, 1988) suggests that the rate of oxidation of Fe^{2+} to Fe^{3+} increases with an increase in pH or concentration of bicarbonate, and the oxidation rate decreases with an increase in the concentration of magnesium, calcium, sulfate, boric acid, and chloride.

An increase in the concentration of the associated species along with an increase in the concentration of a chelator demonstrates the effectiveness of the chelator. The reduced concentration of the single ions as a result of chelation may significantly decrease the ionic activity product for the minerals and avoid precipitation.

Thus, geochemical computer programs can transform analytical data on water composition, temperature, pH, and Eh into meaningful information on the possible consequences of injecting nutrient solutions into the soil. However,

the possible precipitation predicted on the basis of the ionic activity product may not always occur, if inhibited by kinetic reasons.

A literature search was conducted to identify published studies on theoretical and field aspects of the speciation calculations and kinetics of precipitation as related to hydrocarbon contaminated soils and application of fertilizers in soils. The objective of the search was to collect compositional and thermodynamic data necessary for the geochemical modeling of soil-solution interactions expected in a hydrocarbon-contaminated soil subjected to enhanced biodegradation.

The composition of solutions at a contaminated site will be similar to the local groundwater modified by the nutrient solutions. A compilation by White et al. (1963) lists the compositions of groundwaters from several regions of the United States. These groundwaters all contain micronutrients (Mg, K, etc.) and addition of these elements may not be required at most sites for bioreclamation operations in the saturated zone.

Thermodynamic data for calculating chemical speciation (inorganic and organic) are found in the compilations by Smith and Martell (1976) and in the data bases of several geochemical computer programs, such as MINTEQA2 (EPA, 1988), SOLMINEQ88 (Kharaka et al., 1988), and EQ3 (Wolery, 1983). Additional data for aqueous phosphate species are listed in Tardy and Vieillard (1984). All of these sources of data have been acquired.

Formulations of liquid fertilizers commonly include varying proportions of ammonium polyphosphates (Huffman, 1980). The polyphosphates have a higher solubility than orthophosphates and are effective in sequestering metals ions. The sequestering ability of the polyphosphates varies with the chain length. Pyrophosphate sequesters Fe, while longer chain polyphosphates are better for Ca and Mg (Huffman, 1980). Several studies have described the kinetics of phosphate precipitation or nitrogen fixation in agricultural systems. Data in these studies may not be of direct use in our modeling effort, but they do provide insight into possible geochemical behavior of the system.

In addition to kinetics of precipitation, a literature search was conducted on the kinetics of redox processes and polyphosphate hydrolysis. Studies by Millero (1985, 1988) discuss the kinetics of Fe and Cu oxidation.

Review articles by Pankow and Morgan (1981a,b) describe the importance of considering the kinetics of chemical processes for interpreting results of equilibrium calculations. The kinetics of pyrophosphate hydrolysis have been of interest to a variety of researchers in the fields of biological and agricultural chemistry. According to these studies, the half-life (i.e., the time required to hydrolyze 50 percent of the initial pyrophosphate to orthophosphate) of pyrophosphates in natural soils varies from approximately 1 day to several days. Several factors are responsible for the variation in the half-life, including pH, microbial activity, concentration of the enzyme pyrophosphatase, temperature, concentration of other solutes, and the concentration and form of polyphosphate (Gilliam and Sample, 1968; Blanchar and Hossner, 1969; Hossner and Phillips, 1971; Blanchar and Riego, 1976; Frazier and Dillard, 1981). The relatively slow kinetics of polyphosphate hydrolysis may allow for its use as a precipitation inhibitor due to chelation, as a peroxide stabilizer, and as a nutrient source.

A literature search on solution mining was also conducted. The commonly used leach solutions are alkaline, carbonate/bicarbonate solutions with an oxidant (Kuhaida and Kelly, 1978; Alfredson et al., 1978). The oxidant is generally H_2O_2 or dissolved oxygen. One of the problems encountered in solution mining is lowering of permeability by gas blockage. Grant and Burgman (U.S. Patent, 1981) have suggested a method for using ammonium sulfite or bisulfite solution to consume free oxygen and thus increase the permeability. In general, the solution mining literature seems to be of limited applicability to in situ bioreclamation because of the different chemical systems.

SECTION III

GEOCHEMICAL MODELING

A. GEOCHEMICAL MODELING OF EXISTING FORMULATIONS

Geochemical consequences of using existing nutrient formulations for in situ biodegradation were modeled using the computer codes SOLMINEQ.88 (Aggarwal et al., 1986) and MINTEQ. The objective of this modeling effort was to determine the best combinations of chemical parameters and nutrient compositions that may avoid excessive precipitation in the aquifer.

1. Optimal pH and Combination of Salts

Four nutrient formulations from those listed in Table 2 (Section II) were selected to determine their pH (Table 4) by theoretically mixing the proportion of various salts in 1 liter (L) of distilled water. The SOLMINEQ.-88 computer program (Aggarwal et al., 1986) was used for this purpose.

Calculated pHs vary from 5.5 to 9.1, depending upon the buffering capacity, or lack thereof, of the phosphate salts (Table 4). Phosphate or other phase do not appear to be supersaturated in Formulations 1, 2, and 4, indicating that these formulations are chemically stable by themselves. However, formulation 3 is supersaturated with Ca-phosphates (apatite) because of the rather high Ca concentration [16 milligrams per liter (mg/L)].

The pH of Formulation 3 is 6.45. The solubility of Ca-phosphates is quite low at pH 6.45 and decreases with increasing pH. A significant increase in the solubility may occur at low pH values (~4); however, this may not be conducive to microbial growth. The role of Ca in the nutrient formulation is not apparent. Because Ca is not a macronutrient, and is generally present in soils, it is probably best not to use Ca in nutrient formulations to avoid unwanted precipitates.

2. Nutrient Formulations in Groundwater of Varying Compositions

The chemical equilibria calculations presented above were performed with distilled water in the base solution. In field operations, however, local groundwater is used for preparing nutrient solutions. The effect of

TABLE 4. COMPOSITIONS OF NUTRIENT FORMULATIONS USED FOR GEOCHEMICAL MODELING.

Component	Nutrient Concentrations, mg/L			
	I	II	III	IV
NH_4^+	1,683	23	19,584	--
K^+	--	32	--	117
Ca^{2+}	--	0.4	16	100
Na^+	1,216	51	19,284	--
Cl^-	3,319	0.7	28	0.2
NO_3^-	--	78	--	465
SO_4^{2-}	--	9	105,158	96
PO_4^{3-}	2,904	93	52,155	48
CO_3^{2-}	--	57	253	--
BO_3^{3-}	--	--	--	2
Mg^{2+}	--	2	170	24
Mn	0.6	13	0.06	--
Fe	--	0.1	4.5	--
pH*	7.4	9.23	6.45	5.03

References I - Restore 375 manufactured by FMC chemicals
II - Jhaveri and Mazzacca, 1983
III - Raymond et al., 1978
IV - Hoagland's Solution

*Calculated using SOLMINEQ.88

groundwater on the chemical stability of nutrient formulations was evaluated by modeling the equilibria with three groundwater compositions (Table 5). These compositions were chosen to represent a range in pH and total dissolved solids content that may correspond to shallow groundwater from different geographical locations.

Results of theoretically mixing nutrient formulations in groundwaters are shown in Table 6. Groundwater 1 has an initial pH of 6.1 and is not supersaturated with any mineral, except possibly silica. Upon adding the nutrient formulations, the solutions all indicate a potential for the precipitation of Ca-phosphates. The pH of the nutrient solutions is mostly buffered by the nutrient formulations, except for formulation 2 which has the least amount of phosphate buffer and, therefore, low buffering capacity.

Nutrient solutions with Groundwater 2 have slightly higher pHs, reflecting a higher pH of the groundwater (8.4). All solutions are supersaturated with Ca-phosphates. In addition, some silicates and carbonates also are supersaturated because of the higher magnesium/iron (Mg/Fe) and carbonate content of the groundwater.

Mixing nutrient formulations with Groundwater 3 also produced nutrient solutions that are supersaturated with Ca-phosphates and have pH values buffered by the phosphate salts in the formulation.

The mixing calculations indicate that the potential for the precipitation of Ca-phosphate increases when nutrient formulations are prepared in groundwaters, regardless of the variations in the pH or nature of dominant anion/cation of the groundwater. As noted earlier, the solubility of Ca-phosphates decreases with increasing pH in the range of 5 to 9. Since desirable pH for microbial growth is near 7 (Atlas, 1981), it is not possible to decrease significantly the pH of nutrient solutions for increasing the solubility of phosphates.

3. Interaction Between Nutrient Solution and Soil

The calculations for the saturation states of nutrient solutions presented above suggest a potential for the precipitation of phosphate minerals. However, precipitation may not occur in aqueous solutions because of kinetic inhibition. Upon injecting solutions into the aquifer for extended

TABLE 5. GROUNDWATER COMPOSITIONS USED FOR GEOCHEMICAL MODELING OF NUTRIENT FORMULATIONS (from White et al., 1963).

	1*	2*	3*
SiO2	23	28	27
Al	.0		
Fe	.01	.42	.02
Mn	.03		
Ca	6.8	132	3.2
Mg	1.2	16	1.0
Na	2.6	53	262
K	.9	7.6	2.4
HCO3	17	302	149
CO3	0	18	16
SO4	9.0	187	125
Cl	5.0	32	210
F	.1	.3	2.0
NO3	1.2	18	2.5
PO4	.0		.05
pH	6.1	8.4	9.0

* all concentrations are in mg/L, except pH which is in standard units

1: Alluvium, Plymouth, N.H.

2: Alluvium, Gaylord, Kansas

3: Alluvium, Douglas, Arizona

TABLE 6. CALCULATED pH AND SOLUBILITY STATES OF NUTRIENT FORMULATIONS IN GROUNDWATERS OF VARYING COMPOSITIONS.

Nutrient Solution	pH Pure Solution	pH Mixture	Supersaturated Minerals
Groundwater 1	6.1		Silica
+ Nutrient 1	7.4	7.34	Ca-phosphates
+ Nutrient 2	9.23	7.5	Ca-phosphates
+ Nutrient 3	6.45	6.45	Ca-phosphates
+ Nutrient 4	5.03	5.91	Ca-phosphates
Groundwater 2	8.4		(Ca, Fe) Carbonates; Mg-silicate
+ Nutrient 1	7.4	7.4	Ca and Fe phosphates
+ Nutrient 2	9.23	8.35	(Ca, Fe) Carbonates; Mg-silicates; Ca phosphate
+ Nutrient 3	6.45	6.45	Ca phosphates
+ Nutrient 4	5.03	7.5	Ca, Fe Carbonate, Mg-silicate, Ca-phosphates
Groundwater 3	9.0		Ca-phosphate; Mg/Fe silicates, dolomite
Nutrient 1	7.4	7.45	Ca phosphates
Nutrient 2	9.23	9.02	Ca phosphates, dolomite
Nutrient 3	6.45	6.45	Ca phosphates
Nutrient 4	5.03	7.44	Ca phosphates; Mg/Fe silicate, dolomite

periods of time, interactions at the surface of soil minerals will lower the kinetic barrier and induce nucleation of minerals from the supersaturated solutions. In addition, precipitation of phosphates generally occurs in stages with initial precipitation of a metastable phase and its transformation to stable phases. Thus, nutrient solutions that appear chemically stable in nutrient mixing tanks and in short-term, soil-solution mixing experiments may actually result in significant plugging of the aquifer.

a. Extent of Precipitation Based on Geochemical Modeling

Calculations to estimate the extent of precipitation upon injecting phosphate-rich nutrient solutions in a contaminated site can be modeled using data from the Eglin AFB site. The nutrient solutions used at Eglin were prepared by mixing the RESTORE™ 375 formulation with local groundwater. Composition of the feed solutions is given in Table 7 (EAES, 1989).

TABLE 7. CONCENTRATION OF MAJOR COMPONENTS IN REINJECTED GROUNDWATER AT EGLIN.

Component	Concentration (mg/L)
Fe	2.5
Ca	9.0
PO_4	160.0
Cl	400.0
Na	160.0
NH_4	168.0
pH	7.0
Alkalinity (as HCO_3^-)	28.0

The iron content of groundwater from the contaminated region at Eglin is ~12 mg/L (Spain et al., 1989; EAES, 1989), much higher than the concentration of iron in near neutral, shallow groundwaters (<0.1 mg/L; Hem, 1985). The elevated concentration of iron at Eglin is the consequence of reducing conditions resulting from contamination. The iron content of water

used for preparing nutrient solutions was reduced to ~2.5 to 5 mg/L by aeration and sedimentation prior to mixing with nutrients.

The solution composition given above was analyzed for the solubility and extent of precipitation of various minerals using the MINTEQ geochemical modeling code (EPA, 1988). The modeling calculations suggest that the solution is supersaturated with calcium and iron phosphates and with iron oxides and hydroxides. Calculated solubility of Ca and Fe is very low (0.01 mg/L). This low solubility would result in nearly complete removal of these elements by precipitation. Thus, ~19 mg/L of Ca and ~2.5 mg/L of Fe would be available for precipitation. The availability of Fe could be higher due to leaching from the soil under reducing conditions. For the purpose of present modeling calculations, 5 mg/L of Fe was assumed to be available for precipitation.

Assuming that precipitation is not kinetically inhibited, and that phosphate phases are favored over oxide and hydroxide phases, modeling results using MINTEQ indicate that 0.000095 mole of chlorapatite (Ca-phosphate) and 0.00003 mole of vivianite (Fe-phosphate) would precipitate from 1 L of nutrient solution. In addition to Fe and Ca, the precipitate from 1 L will remove nearly 40 mg/L of the orthophosphate from solution and will have a volume of ~0.02 cubic centimeters (cm^3), assuming an average molar volume of 160 cm^3/mole. The calculated precipitate volume would not be significantly different if the pH were lowered to 6.0 (e.g., by an increase in P_{CO2}) or if the Eh were lowered to -100 mV.

Experimental studies of the kinetics of calcium phosphate precipitation (van Kemenade and de Bruyn, 1987) suggest that the stable phase, hydroxyapatite (HAP) or chlorapatite, is preceded by a metastable precursor phase, octacalcium phosphate (OCP) or brushite (DCPD). The solubilities of the precursor phases, OCP or DCPD, are higher than that of HAP (Figure 1). OCP or DCPD precipitated as a precursor phase are transformed upon ageing to HAP by solution-mediated reactions. If we consider for modeling calculations that OCP or DCPD were the only precipitating phases of Ca, the amount of Ca removed by precipitation will be about 1 mg/L, much lower than that calculated above. However, the nutrient injection operations at Eglin were carried out

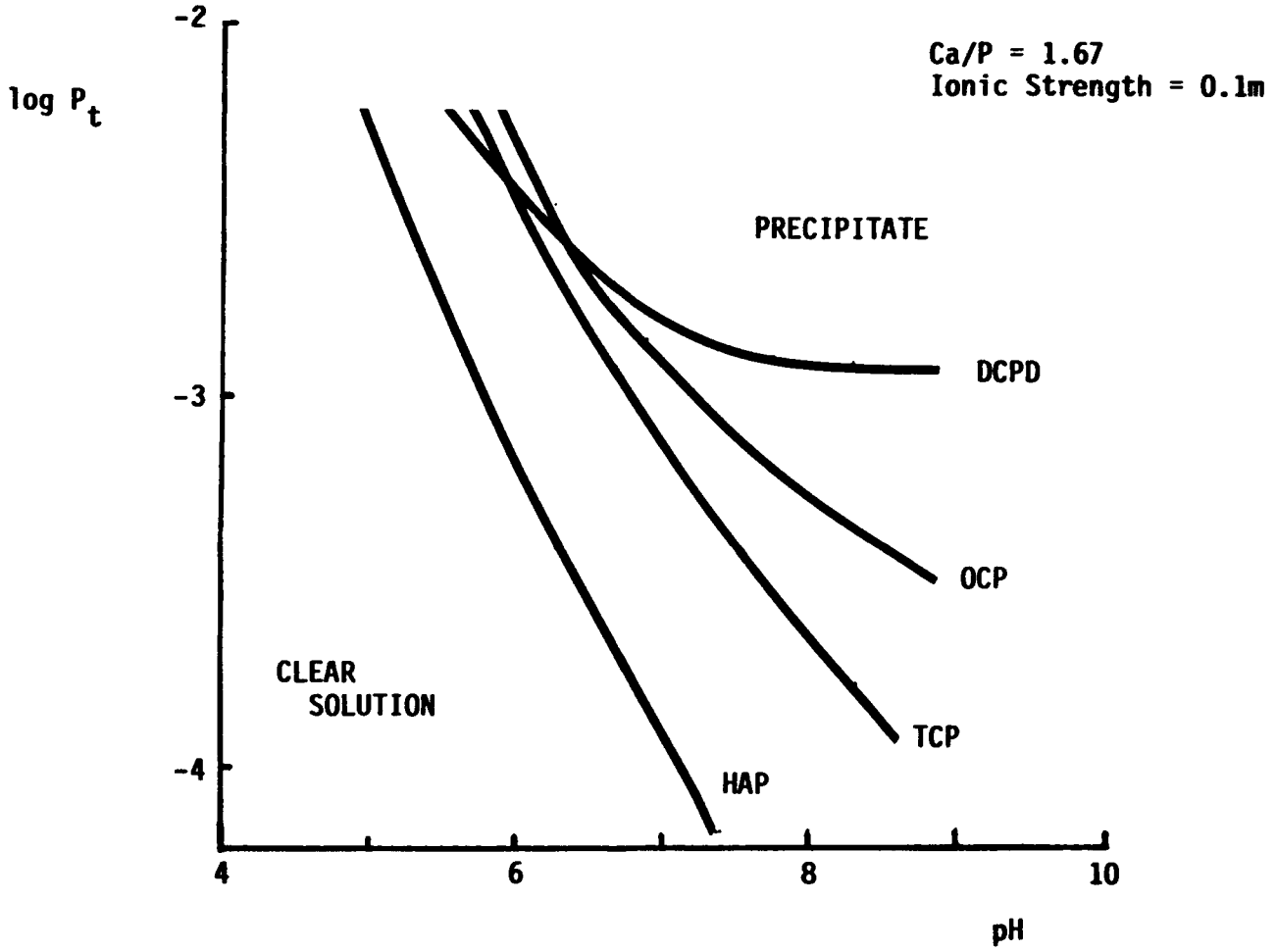


Figure 1. Solubility Isotherms of Phosphate Minerals (After Feenstra and DeBruyn, 1979).

over a period of several months and would most likely have resulted in the transformation of the precursor phase to a stable phase.

The nutrient formulations were injected in the Eglin wells at about 5 gallons per minute (gpm), and the batch application was carried out for a total of 12 hours per week. This amounts to 13,627 L of nutrient solution per week. Based on the volume of precipitate formed per liter of solution (0.02 cm^3), 273 cm^3 of precipitate may be formed in the weekly nutrient injection cycle. For an aquifer with 20 percent porosity, the precipitate from each injection cycle will plug 1,363 cm^3 [0.05 cubic feet (ft^3)] of the aquifer. If precipitation occurs over 50 percent of the pore spaces, the volume of aquifer plugged will be 2,726 cm^3 (0.1 ft^3). In other words, the porosity of a 1-foot x 1-foot patch of the aquifer will be reduced in half to a depth of more than 1 inch in each weekly cycle. Although there is no direct and consistent relationship between porosity and permeability, a significant decrease in porosity will lead to reduced permeability and lower injection rates, if flow paths are not drastically altered.

This analysis of the geochemistry of the aquifer upon nutrient injection agrees with field observations at Eglin in which injection rates in injection wells decreased with time (EAES, 1989) and precipitation of Fe-hydroxides occurred in infiltration galleries. The results based on Eglin data are expected to be valid also for other sites where the Ca and Fe contents of the groundwater are similar. For example, injection rates during enhanced bioreclamation operations near Granger, Indiana, decreased from 40 gpm to 10 gpm within 1 week (API, 1987). The Granger site is located in a silty sand and gravel aquifer. Although water chemistry data are unavailable, precipitation reactions at Granger were probably similar to those at Eglin. Furthermore, the extent of precipitation in sites with limestone lithologies would be expected to be even greater than in sandy lithologies because of the greater amounts of calcium phosphate that would form.

b. Experimental Validation of Modeling Results

Column experiments were conducted to verify modeling predictions for the extent of precipitation. A total of five experiments were conducted

in which Eglin soil was treated with site-specific groundwater containing RESTORE™ 375 or a similar formulation. Three experiments used a formulation similar to RESTORE™ 375, but with no polyphosphates, while the other two used RESTORE™ 375. The concentrations of orthophosphate and ammonium were similar in both sets of formulations (Table 8).

Measured concentrations of orthophosphate during the column experiments are given in Table 9. These data indicate that precipitation of phosphate minerals may have occurred in some of the experiments, while in others the decrease in the phosphate concentrations was minor. As discussed earlier, phosphate minerals precipitate in stages initiated by the formation of a precursor phase. The more soluble precursor phase, generally OCP or DCPD, is then transformed into less soluble, stable phases. The time lag for the initiation of precipitation depends upon the degree of supersaturation of the precursor phase and decreases with increasing supersaturation. Supersaturation is easily expressed in terms of the saturation index (SI) which is defined as follows:

$$SI = \log IAP - \log K \qquad (E\text{-}3)$$

where K is the solubility product (or equilibrium constant) and IAP is the ion activity product for the dissolution reaction of the mineral. For example, the dissolution reaction of DCPD ($CaHPO_4.2H_2O$) is:

$$CaHPO_4.2H_2O + H^+ = Ca^{2+} + PO_4^{3-} + H^+ + 2H_2O \qquad (R\text{-}17)$$

and,

$$IAP = [Ca^{2+}].[PO_4^{3-}].[H^+] \qquad (E\text{-}4)$$

where the square brackets denote the thermodynamic activity of the species. Note that $[H_2O]$ does not appear in the IAP calculation because the activity of water in dilute solutions is ≈1.0. Positive SI values indicate supersaturation and negative SI values suggest undersaturation. When SI = 0, the solution is in chemical equilibrium with the given phase.

The saturation indices of the various phases in experimental solutions also are listed in Table 9. As can be seen from these data, phosphate concentrations did not decrease significantly in solutions that remained undersaturated with the metastable phases (DCPD and OCP; Figure 1). Minor precipitation occurred in solutions that were undersaturated with DCPD,

TABLE 8. NUTRIENT FORMULATIONS USED IN EXPERIMENTS TO VALIDATE MODELING RESULTS.

Component	Concentrations (mg/L) Simulated RESTORE RE-1 uncont.	RE-2 uncont.	RE-7 cont.	RESTORE® 375 RE-8 cont.	RE-9 cont.
Ca^{2+}	9	9	9	13	29
PO_4^{3-}	146	155	150	172	172
$P_3O_5^-$ (as PO_4)	--	--	--	92	92
NH_4^+	168	168	168	168	168
CO_3^{2-}	28	28	28	28	40
Na^+	160	160	160	160	200
K^+	18	18	18	18	18
Fe^{3+}	2.5	2.5	2.5	1	1

TABLE 9. Ca AND P CONCENTRATION AND SATURATION STATES OF PHOSPHATE MINERALS IN SOLUTIONS FROM EXPERIMENTS TO VALIDATE MODELING RESULTS*.

	RE-1					RE-7					RE-8						RE-9					
			SI					SI						SI						SI		
Time (hrs.)	OP	Ca	DCPD	OCP	HAP	OP	Ca	DCPD	OCP	HAP	OP	TP	Ca	DCPD	OCP	HAP	OP	TP	CA	DCPD	OCP	HAP
0	147	9	-0.8	-2.0	2.8	150	9	-0.8	-2.0	2.8	172	264	13	-0.6	0.7	2.9	175	265	29	-0.3	1.0	2.3
2	123					131					174						143	168	5	-0.3	1.7	3.2
4	134					133					173											
24	142	15**	-0.9	-1.2	2.9	108					214	214	14	-0.5	0.2	2.6						
68																	70	84	12	-1.0	-1.5	3.1
96											190	199	25	-0.3	0.6	2.7	62	77	18			
117																	53	65	13			
189						111	47	-0.3	1.4	2.9							41	43	14			
360																	22	20	12			
405																	19	20	12			
453																	19	20	13	-1.8	-4.0	2.72

OP = orthophosphate; TP = total phosphate

* Concentrations of OP, TP, and Ca are in mg/L; Run RE-1 used uncontaminated, Eglin soil and all other runs contained contaminated soil.

** Assumed for modeling purposes

the most soluble phase, but oversaturated with OCP. Solutions in Run RE-9 were supersaturated with DCPD and maximum precipitation occurred in this experiment. After 453 hours, the solution was undersaturated with DCPD and OCP, but not with HAP. This apparent oversaturation with HAP is probably because of the higher solubility of finer-grained phases. The solubility constant used for the SI calculation is for a well-crystallized phase, while the precipitate in the experiment probably is much finer grained. Given sufficient time for crystal growth, the chemical composition of the solution would approach equilibrium with a well-crystallized HAP phase. Consequently, the assumption of thermodynamic equilibrium is likely to be valid for calculating the extent of precipitation in long-term field operations using geochemical modeling techniques.

B. REQUIREMENTS FOR ALTERNATIVE FORMULATIONS

Previous discussions on the decomposition of hydrogen peroxide (Section II) and geochemical modeling suggest two major deficiencies in the existing nutrient formulations: (1) they may lead to excessive precipitation resulting in the plugging of the aquifer, and (2) phosphates alone will not stabilize hydrogen peroxide in the presence of enzymatic catalysts. Since phosphate is used as a nutrient as well as a peroxide stabilizer, and it is also a major source of precipitate, the existing formulations should to be modified primarily to change the phosphate content.

The maximum concentration of orthophosphate necessary to avoid excessive precipitation from nutrient solutions (pH ≈7) is ~10 mg/L. However, higher injection concentrations may be required to stimulate downgradient biodegradation and to improve hydrogen peroxide stability.

1. Microbial Phosphate Requirements

Orthophosphate concentrations necessary only to stimulate microbial growth in the aquifer can be estimated given the appropriate growth constants and related parameters. Nutrient requirements in the subsurface are determined by two processes: transport of solutes and growth of microbial colonies where oxygen generally is the limiting nutrient. There are data available

that can be used to estimate the limiting concentration of a nutrient when all other nutrients are in excess and not limiting the rate of microbial growth. However, it is presently not possible to estimate the minimum, non-limiting concentrations of one nutrient when some other nutrient is limiting microbial growth. Consequently, the concentration of orthophosphate just above the level where it will create P-limiting conditions can not be estimated for systems where oxygen is the limiting nutrient.

An approximation for the maximum level of orthophosphate corresponding to oxygen consumption under oxygen-limiting conditions can be derived as follows. Assume that each mole of oxygen consumes 1 mole of carbon to produce biomass that has a C:N:P ratio of 100:10:1 (Atlas, 1981). Then, consumption of each mole of oxygen will require 0.01 moles of P as nutrients. For 300 mg/L (~0.01 moles/L) oxygen, only 9 mg/L of available orthophosphate may be required. This is a maximum concentration corresponding to an instantaneous use of oxygen; concentration of phosphate to avoid P-limiting conditions may be even lower. Data in Table 10 show that naturally available phosphate in Eglin AFB soils (~18 mg/kg) was sufficient to provide non-P-limiting growth conditions.

TABLE 10. MICROBIAL ENUMERATIONS IN EGLIN AFB SOIL (UNCONTAMINATED). MINERAL SALTS AGAR WITH VARYING KH_2PO_4.

KH_2PO_4 (mg/L)	Enumeration (CFU/g, dry wt.)
0	2.4×10^6
0.005	2.4×10^6
0.05	2.4×10^6
0.5	2.2×10^6

2. Phosphate Requirement for Peroxide Stabilization

Existing nutrient formulations contain excessive orthophosphate to decrease the rate of peroxide decomposition. This role of phosphate in the nutrient solution is rather limited and may only inhibit the inorganic catalysts of peroxide decomposition. As noted earlier, peroxide stabilization has not been achieved in the field using excess orthophosphate. Thus, the phosphate concentration may be lowered to meet only the microbial requirements and stabilization of peroxide may be achieved by adding other inhibitors of peroxide decomposition. If orthophosphate is the only stabilizer used, its concentration may be based on the amount required to sequester the expected aqueous Fe species in the aquifer.

3. Alternative Formulations

a. Phosphate Source

An alternative source of phosphate in nutrient formulations is polyphosphate. While polyphosphates are mentioned infrequently in the bioreclamation literature, it is known that they have been used at some bioreclamation sites (Brown, 1989). Polyphosphates of varying chain lengths [e.g., pyrophosphate (P_2O_7), tripolyphosphate (P_3O_{10}), etc.], hydrolyze to provide the nutrient form of P, orthophosphate, through intermediates having successively lower chain lengths. For example, the hydrolysis of tripolyphosphate is represented by the following two reactions:

$$P_3O_{10}^{5-} + H_2O \rightarrow P_2O_7^{4-} + PO_4^{3-} + 2H^+ \qquad \text{(R-18)}$$

$$P_2O_7^{4-} + H_2O \rightarrow 2PO_4^{3-} + 2H^+ \qquad \text{(R-19)}$$

Upon complete hydrolysis, each mole of tripolyphosphate provides 3 moles of orthophosphate. The kinetics of the hydrolysis reactions are influenced by several factors. Most important to biodegradation are pH, the ionic composition of the solution, microbial activity, concentration of the enzyme polyphosphates, and chain length. The half-lives of Reactions R-26 and R-27 in natural soils are found to vary from approximately 1 day to tens of days. Thus, polyphosphates can in effect be used as an "in situ," slow-release source of orthophosphate. The rate of orthophosphate production can

be "geochemically" controlled to avoid the plugging problems. Polyphosphates also have the following characteristics that make them an attractive constituent of nutrient solutions:

- They have a higher solubility than orthophosphate, thus allowing their use in higher concentrations without plugging problems.
- They inhibit the crystallization of iron hydroxides and calcium carbonate and are effective chelators for the major cations, Fe, Ca, and Mg.
- They adsorb on the soil much less than the orthophosphates.
- They may have a stabilizing effect on hydrogen peroxide.
- They are not significantly more expensive (e.g., sodium pyrophosphate is approximately 1½ times the cost of phosphate).

b. Other Constituents

In addition to phosphate, the nutrient formulations contain a nitrogen source with or without micronutrients. There are no apparent complications caused by any of the other nutrients in existing formulations. Thus, we recommend that ammonium chloride at a concentration of ~160 mg/L (as in RESTORE™ 375) be used as a nitrogen source. Specific micronutrients may be added in trace quantities if they are not already present in the groundwater.

Finally, peroxide stabilizers may be added to decrease the rate of hydrogen peroxide decomposition. Optimum concentrations of these additives will be estimated on the basis of experimental studies described in the following section.

SECTION IV

EVALUATION OF ALTERNATIVE NUTRIENT FORMULATIONS

A series of bench-scale experiments was conducted to evaluate the effectiveness of alternative nutrient formulations in decreasing or eliminating the problems of plugging and rapid hydrogen peroxide decomposition. The bench-scale experiments described in this section are divided into the following categories:

(1) Performance of Polyphosphates: Batch and column studies to evaluate the performance of polyphosphates as a source of nutrient phosphorus and in minimizing plugging.

(2) Peroxide Stabilization: Batch studies to evaluate the effectiveness of various additives in decreasing the rate of peroxide decomposition.

(3) Nonbiological Oxygen Demand: Batch studies to evaluate inorganic, oxygen-consumption reactions.

(4) Aquifer Simulation: Aquifer-simulator studies to verify, on a larger scale, conclusions based on batch and column studies.

A test plan was developed prior to beginning the experimental studies. This plan (Appendix A) presents a discussion of the bases on which specific experimental studies were designed. The original test plan was altered during the course of the work based on the results of early experiments. Major deviations from the original test plan included the following:

(1) Addition of trimetaphosphate (TMP) to the group of polyphosphates to be evaluated. It was initially planned to use only pyro- and tripolyphosphates for experimental evaluation. However, it became apparent that these two classes of polyphosphates may not be good alternatives to orthophosphate because of their relatively rapid hydrolysis and significant adsorption.

(2) Use of Homestead soil for batch studies only because of a lack of permeability in column beds.

(3) Conduct of additional experimental studies to examine adsorption of polyphosphates.

A. PERFORMANCE OF POLYPHOSPHATES

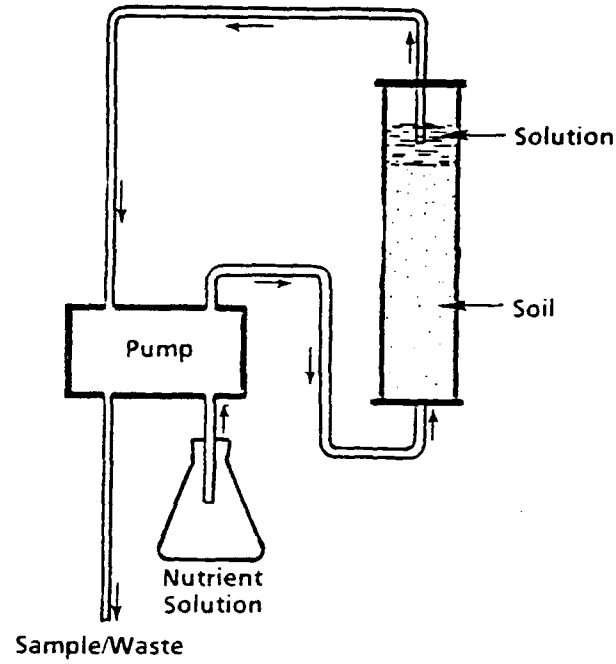
Phosphorous in the orthophosphate form is an essential nutrient for microbial growth. The various polyphosphate species hydrolyze in aqueous solutions to produce orthophosphate and, therefore, can be used in nutrient formulations. Because of the higher solubility of polyphosphates compared with orthophosphates, polyphosphate-based nutrient formulations may alleviate plugging of the aquifer due to excessive precipitation.

The availability of orthophosphate from the polyphosphates is a function of the rate of polyphosphate hydrolysis which varies in different soils depending upon several factors, including pH, soil composition, and microbial activity. Three polyphosphates, pyrophosphate, tripolyphosphate and trimetaphosphate, were chosen to assess the performance of polyphosphate-based formulations in decreasing the plugging problems while providing sufficient concentrations of nutrient phosphate. Contaminated and uncontaminated soils from the Eglin AFB and uncontaminated soil from the Homestead AFB were selected for the polyphosphate studies to evaluate the effects of soil chemical composition, soil microbial activity, pH, and temperature on the performance of polyphosphates in nutrient formulations.

1. Experimental Design

Both batch and column experiments were conducted to examine polyphosphate hydrolysis. Batch experiments involved the mixing of soil and solution in glass reaction vessels. The vessels containing the appropriate reaction mixture were then placed on a mechanical shaker to facilitate maximum reaction. Samples were withdrawn periodically with a syringe and filtered or centrifuged.

Column experiments were conducted using pharmaceutical columns made by the Pharmacia Company. Both single pass and recirculatory modes were used (Figure 2). When contaminated soil was used, the columns were aerated frequently with room air to maintain aerobic conditions in the soil. The direction of flow in the columns was from bottom to top and the flow rate was maintained to provide a residence time of 60 to 90 minutes.



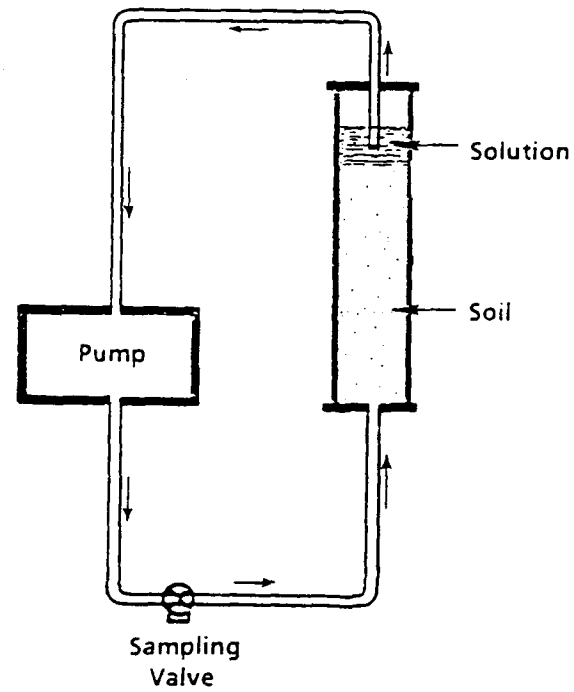


Figure 2. Schematic Diagram Showing the Design of Column Experiments For Polyphosphate Hydrolysis.

2. Soil Characteristics

Soils from the Eglin and Homestead Air Force Bases were used for the polyphosphate experiments. X-ray diffraction analyses indicate that the Eglin soils consist primarily of quartz (Appendix B). Partial chemical analyses of these soils (Table 11) are consistent with the X-ray analysis. The minor amounts of P, K, Ca, Mg, and sodium (Na) in the Eglin soil may be contained in minerals present in trace quantities that may not be identified by X-ray diffraction patterns of bulk soil. Iron (Fe) in Eglin soils may be present in clay minerals (such as glauconite) that were not identified by X-ray diffraction. In addition, Fe in the uncontaminated soils is present as Fe-hydroxide (probably amorphous) coatings on sandy grains which impart a yellowish color to the soil.

Microbial activity in the Eglin soils was characterized by agar plating. The uncontaminated Eglin soils have a much higher microbial activity (2.4×10^6 colony-forming units/gram [CFU/g], dry weight) compared with the contaminated soils (2.4×10^4 CFU/g, dry weight). This is probably because of the anaerobic conditions that result from contamination.

X-ray diffraction pattern of the Homestead soil indicates that these soils contain a mixture of calcite and aragonite with minor quartz (Appendix B). Relatively high Mg and Fe contents of these soils compared to the Eglin soils (Table 11) are probably due to the presence of Fe and Mg in calcite and aragonite.

3. Pyrophosphate and Tripolyphosphate Hydrolysis

Experiments to study the hydrolysis of pyrophosphate and tripolyphosphate were conducted with Eglin and Homestead, abiotic ($HgCl_2$ treatment) and natural soils. Both uncontaminated and contaminated soils from Eglin AFB were used. The abiotic soils were used to differentiate the roles of inorganic and biological parameters on the hydrolysis of polyphosphates. Polyphosphate solutions were prepared by adding an appropriate amount of sodium pyrophosphate (Reagent Grade; Sigma Chemical Co.) or sodium tripolyphosphate (Practical Grade (97%); Sigma Chemical Co.) to site-specific groundwaters (Table 12) or deionized water.

TABLE 11. CHARACTERISTICS OF SOILS USED IN EXPERIMENTAL STUDIES.

	Unit	Eglin		Homestead
		Uncontaminated	Contaminated	Uncontaminated
pH (in situ)	standard	6.6	5.5	7.6
Organic matter	%	.2	<.2	2.0
Microbial population	CFU/gm (dry)	$2.4x10^6$	$2.4x10^4$	2.0
Exchangeable				
N - NH_4	mg/kg	<6.7	<6.7	<6.9
N - NO_3	mg/kg	<2.5	9.5	<2.5
K	mg/kg	10.5	13.0	31.0
PO_4	mg/kg	18.6		21.3
Total metals				
P		52	18	16.0
K	"	140	59	117
Ca	"	116	184	304705
Mg	"	138	43	1111
Fe	"	1639	357	3648
Na	"	22	30	430
Cu	"	1.0	<0.7	4.9
Zn	"	5.9	5.8	4.3
Mn	"	8.6	3.6	48.2
B	"	5.1	4.3	16.6

TABLE 12. PARTIAL CHEMICAL COMPOSITION OF GROUNDWATER USED IN EXPERIMENTAL STUDIES.

	Concentration (mg/L) Eglin	Homestead
Ca	22.2	100.4
Fe	0.1	<0.05
P	0.1	0.16
Alkalinity as (HCO_3^-)	28.0	N.A.
pH	6.32	7.54

a. Abiotic, uncontaminated soils

Dissolved orthophosphate concentrations over a period of 3 days from batch experiments with abiotic soils are listed in Table 13. Less than five percent of the pyrophosphate may have hydrolyzed to orthophosphate after 3 days in contact with abiotic, Eglin soil. In abiotic Homestead soil, no orthophosphate was detected in any of the samples. These results suggest that either the rates of pyrophosphate and tripolyphosphate hydrolysis in abiotic soils were very slow or that significant sorption (adsorption and precipitation) of phosphates may have occurred during the experiment.

Total phosphate concentrations of the final solutions (Table 13) indicate that low orthophosphate concentrations in the batch experiments were due to the removal of phosphates from solution by sorption (adsorption and precipitation) on the soil. Sorption removed ~75 percent of the phosphates in experiments with the Eglin soil and ~100 percent in experiments with the Homestead soil. Both orthophosphate and pyrophosphate were being sorbed on the Homestead soil while a significant proportion of the remaining phosphate in contact with the Eglin soil was pyrophosphate. Thus in experiments with

the Eglin soil, some of the pyrophosphate hydrolyzed to orthophosphate, which then was removed by sorption, although sorption of pyrophosphate may also have occurred.

TABLE 13. PYROPHOSPHATE HYDROLYSIS IN STERILIZED EGLIN AND HOMESTEAD SOILS.

pH (Initial)	Orthophosphate* (mg/L)			Total Phosphate* (as mg/L PO_4)		Final pH
	1 day	2 days	3 days	Initial	3 days	
Eglin Soil						
5	6.9	13.4	5.5	190	30.5	5.70
7	5.5	7.6	6.7	190	47.8	6.41
9	5.0	7.1	9.1	190	40.5	7.41
Homestead Soil						
5	<0.1	<0.1	<0.1	190	<0.1	7.54
7	<0.1	<0.1	<0.1	190	<0.1	7.50
9	<0.1	<0.1	<0.1	190	<0.1	8.03

* remaining in solution

For polyphosphates to be used successfully in nutrient formulations, the sorption of phosphates on the soils must be characterized. Therefore, we limited future experiments to natural soils only so as not to alter the surface properties by pretreatment with $HgCl_2$. In addition, experiments with Homestead soil were conducted only at a pH near 7.6 to minimize the dissolution of soil as evidenced by a shift of up to ~2 pH units in the batch experiments described above (Table 13).

b. Natural, uncontaminated Soils

Results of batch experiments of pyro- and tripolyphosphate hydrolysis with natural, uncontaminated soils from the Eglin and Homestead AFBs are given in Tables 14 and 15, respectively. These data again indicate that pyrophosphate and tripolyphosphate were sorbed significantly on the

TABLE 14. PYROPHOSPHATE HYDROLYSIS IN EGLIN AND HOMESTEAD SOILS.

pH (initial)	Orthophosphate* (mg/L)			Total Phosphate* (as mg/L PO_4)	
	1 day	2 days	4 days	Initial	4 days
Eglin					
5	<1	2.9	<1	19.0	<1
7	<1	1.5	<1	19.0	<1
9	<1	<1	1.0	19.0	1.5
Homestead					
7	<1	<1	<1	19.0	<1

* remaining in solution

TABLE 15. TRIPOLYPHOSPHATE HYDROLYSIS IN EGLIN AND HOMESTEAD SOILS.

pH (initial)	Orthophosphate* (mg/L)			Total Phosphate* (as mg/L PO_4)	
	1 day	2 days	4 days	Initial	4 days
Eglin					
5	5.5	2.1	2.3	142.5	6-7
7	20.6	23.7	38.1	142.5	49.4
9	22.1	40.0	52.4	142.5	72.4
Homestead					
7	<1	<1	<1	142.5	<1

* remaining in solution

soils. Presence of some orthophosphate in the daily samples from the Eglin soil experiment suggests that hydrolysis of the polyphosphates did occur before sorption. However, sorption of phosphates (ortho and poly) on Homestead soil was very rapid because no orthophosphate was detected in any of the 1, 2, or 4 day samples. Because of the extensive sorption on Homestead soil, additional experiments to evaluate the performance of polyphosphates were conducted using only the Eglin soil. Polyphosphate solutions in these experiments were prepared using deionized water instead of site-specific groundwater to lower the potential for phosphate precipitation. Results of batch experiments with deionized water are listed in Tables 16 and 17, respectively. These results indicate that using deionized water did decrease the sorption of phosphates on the soils and that tripolyphosphate may be a possible alternative for use in nutrient formulations.

The potential performance of tripolyphosphate in the field was evaluated using column experiments. In these experiments, uncontaminated or contaminated Eglin soils were treated with TPP-spiked (170 mg/L as PO_4) Eglin groundwater in a recirculatory mode.

In the uncontaminated soil experiment, nearly all of the TPP was hydrolyzed to orthophosphate in 4 days. The total phosphate concentration at the end of the experiment decreased to ~100 mg/L (as PO_4) because of the sorption of orthophosphate.

In contact with the contaminated soil, hydrolysis of TPP was much faster and nearly 90 percent of the phosphate was removed from solution within 2 hours. The contaminated soil experiment was repeated using a single-pass mode, instead of recirculatory mode, with a residence time of ~90 minutes in the soil. Results of the single-pass experiment (Table 18) show that the total phosphate concentration in the effluent reached nearly the same value as that of the influent in ~3 hours; the less than 3-hour values were lower because of mixing with some of the deionized water used for filling the soil into the column. Orthophosphate concentration in hourly samples from 3 to 6 hours was nearly constant at ~40 mg/L. Total phosphate concentration in the effluent did not decrease significantly, indicating only negligible sorption of phosphates on the soil in the short residence time of ~90 minutes.

TABLE 16. PYROPHOSPHATE HYDROLYSIS IN UNCONTAMINATED EGLIN SOIL.

pH		Orthophosphate* (mg/L)			Total Phosphate* (as mg/L PO_4)	
(Initial)	Final	3 days	4 days	5 days	Initial	Final
5	7.2	14.6	14.0	14.0	38.0	29.7
9	7.3	5.2	6.3	6.6	38.0	23.3

* remaining in solution

TABLE 17. TRIPOLYPHOSPHATE HYDROLYSIS IN UNCONTAMINATED EGLIN SOIL.

pH		Orthophosphate* (mg/L)				Total Phosphate* (as mg/L PO_4)	
Initial	Final	1 day	3 days	4 days	5 days	Initial	Final
5	6.9	4.1		7.2	6.9	28.5	9.4
9	7.0	2.0		7.4	10.4	28.5	14.5

* remaining in solution

TABLE 18. HYDROLYSIS OF TPP IN CONTAMINATED EGLIN SOIL (COLUMN EXPERIMENTS IN SINGLE PASS FLOW WITH ~90 MIN RESIDENCE TIME IN THE SOIL).

Time (h)					
Total	In contact with soil	OP	TP	Ca	Fe
0	0	<0.1	260	9.6	
2	1.5	24.5	175.0		
3	1.5	35.0	268.8	38.5	19.7
4	1.5	38.4	220.0		
4.5	1.5	41.9	220.6		
5.5	1.5	52.4	274.4	33.7	11.8
New phosphate solution added after batch mode overnight					
0	0	<0.1	285		
1	1.5		182		
2	1.5	42.4	299	34.6	6.4
3	1.5	44.3	291		
4	1.5	44.7	297	30.9	5.0

OP = Orthophosphate
TP = Total phosphate
All concentrations are in mg/L

The extent and mechanism of sorption of each of the phosphate species (poly and ortho) in the batch and column experiments was investigated with several short-term experiments. Results of these experiments (Table 19) indicate that in Eglin soils, orthophosphate is the only species that adsorbs significantly. The polyphosphates show negligible sorption; differences in the measured concentrations at 1 and 2 hours are within the error of measurement.

On the other hand, all of the phosphate species are sorbed on the Homestead soil. Orthophosphate is removed from the solution within 15 minutes and significant proportions of the polyphosphates are removed within 1 hour. Phosphate removal in contact with the Homestead soil occurs due to both adsorption and precipitation of Ca phosphates (Table 20).

4. TMP Hydrolysis

Tripolyphosphate and pyrophosphate hydrolysis data presented above suggest relatively rapid rates of hydrolysis and removal from solution by sorption. Even though the performance of tripolyphosphate seems to be better than pyrophosphates, it may not be the best alternative for orthophosphate, particularly in calcareous soils. Therefore, the performance of a third polyphosphate species, trimetaphosphate (TMP, $P_3O_9^{-3}$), was evaluated.

TMP contains PO_4 groups linked in a ring structure, as opposed to linear linkages in tripolyphosphate and pyrophosphate. Because of its structure, TMP adsorbs on soils to a lesser extent and has a higher solubility compared with TPP and PP.

The feasibility of using TMP in nutrient solutions was first evaluated by conducting adsorption experiments, as described earlier for TPP and PP. The adsorption data (Table 21) suggest negligible adsorption of TMP on Eglin soil. For Homestead soil, adsorption was relatively minimal in 2 hours. However, more than 50 percent of the total phosphate was removed from the solution in 18 hours. Precipitation was suspected to be of lesser or no importance because of the higher solubility of TMP. However, TPP and PP produced by the hydrolysis of TMP may have precipitated, lowering the total phosphate content. Results of the short-term experiments indicated that TMP may provide a workable alternative for nutrient source in calcareous soils.

TABLE 19. ADSORPTION OF PHOSPHATES ON EGLIN AND HOMESTEAD SOILS.
SOIL:WATER = 1:5.

Soil	pH (Initial)	Concentration (mg/L) t = 0 OP	t = 0 TP	15 min OP	15 min TP	1 hr OP	1 hr TP	2 hr OP	2 hr TP	3 hr OP	3 hr TP
Orthophosphate											
Eglin	5	9.5				7.6		5.1		5.0	
Homestead	8	9.5		<1							
Pyrophosphate											
Eglin	5	<1	19			<1	16.2	<1	15.3		
	9	<1	19			<1	15.5	<1	15.2		
	7*	<1	190				191.2		182.3		
Homestead	8	<1	19			<1	<1	<1	<1		
	8*		190		114.8		82.7				
Tripolyphosphate											
Eglin	5	<1	28.5			<1	24.9	<1	24.8		
	9	<1	28.5			<1	28.2	<1	25.1		
	8*		285				259.4				
Homestead	8*		285		203.2		166.5				

* soil/water ratio = 1:10
OP = Orthophosphate
TP = Total Phosphate as PO_4

TABLE 20. SHORT-TERM SOIL-SOLUTION INTERACTION USING THE HOMESTEAD SOIL.

Solution	Concentration (mg/L)					
	t = 0 min			t = 10 min		
	Ca	Fe	PO_4 (total)	Ca	Fe	PO_4 (total)
1×10^{-4} m PO_4	<0.05	<0.05	9.5	85.1	<0.05	0.6
5×10^{-4} m TPP	<0.05	<0.05	142.5	75.1	<0.05	101.1
Deionized H_2O	<0.05	<0.05		<0.05	<0.05	<0.05

a. Natural, uncontaminated soil

Batch experiments for TMP hydrolysis were conducted using site-specific groundwaters and uncontaminated Eglin and Homestead soils. These experiments were conducted only at the pH of natural groundwaters (6.6 to 7 for Eglin and 7.6 for Homestead) to avoid significant soil-solution reaction.

In experiments with the Homestead soil, nearly all of the phosphate was removed from the solution within 90 minutes. Lower sorption in the short-term experiments reported in Table 21 probably was because of a higher (1:10) soil/solution ratio. Consequently, any of the phosphate species (ortho or poly) may not be used in nutrient formulations for biodegradation operations in calcareous soils.

Results of batch experiments of TMP hydrolysis in Eglin soil are given in Table 22. Sorption of phosphates on the Eglin soil was very minor and the data in Table 22 indicate a rather slow rate of hydrolysis.

b. Contaminated Eglin Soil

The potential performance of TMP in the field was evaluated with a column experiment using contaminated soil, as described earlier for TPP. The results of this experiment are given in Table 23 and are similar to those obtained for TPP.

TABLE 21. ADSORPTION OF TMP ON UNCONTAMINATED, EGLIN AND HOMESTEAD SOILS. SOIL/WATER RATIO = 1:10(v/v).

	Time (min)	Concentration (mg/L) 1 OP*	1 TP**	2 OP*	2 TP**
Eglin	0	2.50	243.0	<1	29.4
	60	2.53	243.0		30.0
	120	2.23	237.2		28.0
	240	2.45	224.2		29.4
	480	3.09	240.0		26.0
	1080	6.62	257.6		30.1
Homestead	5		243.0		29.4
	15		227.9		24.8
	30		215.1		25.9
	60		227.9		29.0
	90		190.5		17.5
	120		180.2		17.6
	1080		126.2		<2.0

* OP = orthophosphate
** TP = total phosphate expressed at PO_4

TABLE 22. TMP HYDROLYSIS IN UNCONTAMINATED EGLIN SOIL. SOIL/WATER RATIO = 1:10(v/v).

Time (h)	Concentration (mg/L)	
	OP	TP
0	<1.0	28.1 (Ca = 22.2, Fe = 0.1)
25	<1.0	21.5 (Ca = 43.6, Fe = 0.2)
49	1.0	20.9
141	4.6	19.5
165	6.3	19.1

TABLE 23. HYDROLYSIS OF TMP IN CONTAMINATED EGLIN SOIL (COLUMN EXPERIMENTS IN A RECIRCULATORY MODE WITH ~90 MIN RESIDENCE TIME IN THE SOIL).

Time (h)	TMP hydrolysis			
	OP*	TP**	Ca	Fe
0	2.7	263.1	9.6	
2	2.3	102.0		
4.5	15.0	N.A.		
6.5	27.3	65.2		
23	29.4	35.3	90	0.7
25	28.7	34.2	88.7	0.9
New phosphate solution added				
1	30.1	36.6	94.4	0.3
3	37.2	86.2	65	1.2
5	49.6	86.5		
22	48.8	69.3		
25	55.6	63.9		

* OP = orthophosphate concentration (mg/L)

** TP = total phosphate expressed as mg/L PO_4

5. TMP Hydrolysis at Higher Temperature

The hydrolysis of polyphosphates in soils is catalyzed by the various polyphosphatase enzymes (pyrophosphates, tripolyphosphatase, trimetaphosphatase, etc.). Since the rates of chemical and biochemical reactions are temperature dependent, the hydrolysis of TMP was evaluated at an elevated temperature of 35°C. The experimental design was similar to that of column experiments described earlier. The column was enclosed in a jacket with two ports allowing water to flow in between the jacket and the column. Elevated temperature in the column was achieved by continuous circulation of hot water maintained at 35°C in a constant-temperature water bath. Uncontaminated Eglin soil was used and the TMP-spiked synthetic groundwater was injected in a single-pass mode. The residence time of the solution in the soil bed was ~60 minutes. Results of the higher temperature experiment are given in Table 24. The flow was maintained in the single-pass mode for 4 hours and then in the recirculatory mode overnight. Sorption of phosphates was minimal in the 16-hour period. The experiment was repeated by introducing new solution again in the morning. The flow was in a single-pass mode for 4 hours and recirculated for 2 hours. Sorption of phosphate again was minimal.

The hydrolysis data in Table 24 suggest that the rates of TMP hydrolysis are not affected by temperature variations in the range of 25 to 35°C. This is consistent with other studies of polyphosphate hydrolysis (e.g., Dick and Tabatabai, 1986) which also indicate that temperature changes from 20 to 30°C will have only a minor effect on rates of polyphosphate hydrolysis.

6. Rate Constants for Polyphosphate Hydrolysis

Polyphosphate hydrolysis in aqueous systems generally follow first order kinetics (Pankow and Morgan, 1980):

$$\frac{dC}{dt} = -K\,C \qquad \text{(E-5)}$$

where C is the concentration of the polyphosphate species, t is time, and K is the rate constant. The concentration of unhydrolyzed polyphosphate at a time t can be obtained by integrating the above equation:

$$\ln C = \ln C_o + K\,t \qquad \text{(E-6)}$$

TABLE 24. TMP HYDROLYSES AT ELEVATED TEMPERATURE (35°C) IN UNCONTAMINATED EGLIN SOIL.

Time (h) total	in contact with soil	Orthophosphate (mg/L)	Total Phosphate (mg/L)
Single-pass mode with 30 min residence time			
0	0	2.4	280
1	0.5	<1	272.9
2	0.5	<1	233.1
3	0.5	12.4	240.1
4	0.5	10.0	255.9
(Recirculated)			
20	16	123.5	247.6
Restarted with single-pass mode			
0	0	2.5	270.9
1	0.5	11.4	272.9
2	0.5	3.9	240.03
3	0.5	8.3	247.6
4	0.5	8.2	241.5
(Recirculated)			
5	0.5	16.0	252.5
6	1.5	21.56	255.9

Accordingly, differential equations for the hydrolysis of the various phosphate species are:

$$d(TPP)/dt = -K_1 (TPP) \quad \text{(E-7)}$$

$$d(PP)/dt = -K_2 (PP) + K_1 (TPP) \quad \text{(E-8)}$$

$$d(P)/dt = K_1 (TPP) + 2 K_2 (PP) \quad \text{(E-9)}$$

The hydrolysis of TMP results in the production of TPP followed by the hydrolysis of TPP given by the above equations.

Concentrations of the polyphosphates measured in batch and column experiments described in this section were used to estimate rate constants for PP, TPP, and TMP hydrolysis. Detailed discussion of the methods for these calculations is given in Appendix C.

Calculations based on data from the batch experiments result in hydrolysis rate constants of 3.0×10^{-6} sec^{-1} for PP, 1.5×10^{-4} sec^{-1} for TPP, and 2.0×10^{-6} sec^{-1} for TMP. The batch experiments were conducted with uncontaminated Eglin AFB soil. Data from the single-pass column experiments, which used contaminated Eglin soil, give higher values, 2.0×10^{-3} sec^{-1} for TPP and 2.0×10^{-5} sec^{-1}. These differences are likely due to (1) lower soil/solution ratios in batch experiments and/or (2) lower microbial population in contaminated soils.

Previous studies of polyphosphate hydrolysis (Dick and Tabatabai, 1986) have not attempted to estimate the value of individual rate constants for the hydrolysis of TPP and TMP. Instead a composite rate constant for the production of orthophosphate has been given. This rate constant, K', can be estimated by plotting the log concentration of orthophosphate produced or of the nonhydrolyzed polyphosphates versus time. Alternatively the K' can be calculated by the equation

$$K'(sec^{-1}) = \ln(3 - P/C)/t \quad \text{(E-10)}$$

where C is the initial concentration of TPP or TMP. Column experiment data obtained in this study give a composite rate constant of 1.8×10^{-4} sec^{-1} for TPP and TMP hydrolysis, higher than the values given by Dick and Tabatabai (1986). These differences also may be explained by differences in soil characteristics. Dick and Tabatabai used soils with relatively higher clay content (>27 percent), compared with almost no clay content in the soils used

in this study. A higher clay content may result in increased sorption and lower microbial activity and, therefore, apparently lower rates of polyphosphate hydrolysis. Better agreement with previous studies is obtained when rate constants based on single-pass column experiments are compared.

B. HYDROGEN PEROXIDE DECOMPOSITION AND STABILIZATION

The objective of this group of experiments was to study the effect of various additives identified in the literature search for decreasing the rate of hydrogen peroxide decomposition in the soil. Peroxide decomposition in the soil is catalyzed primarily by the enzyme catalase and, to a lesser extent, by multivalent elements such as Fe and Mn. The experiments described in this section were designed to evaluate the effect of additives on both enzymatic and inorganic catalysts.

1. Experimental Design

Batch experiments were used to study hydrogen peroxide stabilization. As noted in the initial test plan, the approach of Lawes (1988) was to be used. In this approach, the soil is placed in an Erlenmeyer flask, the peroxide solution is added, and the flask is quickly stopper with a one-hole stopper. A glass tube from the stopper hole is connected to a latex tubing. The other end of the latex tubing is placed at the closed end of a water-filled inverted buret kept in a jar of water. The decomposition of peroxide in the flask produces oxygen which escapes through the glass tube into the buret. Displacement of water in the buret provides a measure of the extent of peroxide decomposition.

This design was used in several preliminary experiments; however, inconsistent and unreliable results were obtained. The principal sources of error were considered to be (1) an incomplete reaction and transfer of oxygen from the flask to the buret and (2) the method used to measure peroxide decomposition is inherently less sensitive to small differences in the extent of decomposition. Consequently, a new design was used based on a combination of the designs for column and batch experiments. A column was packed with soil (15 to 25 grams) and the pretreatment solution, if used, was passed through the soil for 30 to 80 minutes. The pretreatment solution consisted of the

nutrient solution spiked with a peroxide-stabilizing additive. Following pretreatment, the soil was drained of the residual solution and 10 mL of peroxide-containing nutrient solution (with stabilizing additive) were injected. The column was sealed off and left in the batch reaction mode for 1 hour. Finally, the solution was sampled and peroxide measured by a ceric acid titration procedure using the appropriate HACH® kit. This modified design did not result in a perfect solution to problems of incomplete soil-solution reaction. Oxygen formed by rapid decomposition of peroxide in the lower part of the column pushed the solution in the rest of the column above the soil, preventing reaction. In addition, the lower part of the column became impermeable because of gas blockage. Thus the sample taken at the end of the experiment was from the incompletely reacted, supernatant solution.

The rapid decomposition of peroxide was likely due to a lack of acclimation of soil microbes to the added peroxide. Thus, the experimental design was further modified to provide for acclimation. In this design the peroxide solution was not introduced in a single batch, but was passed through the soil for 60 to 80 minutes. Flow was then stopped and the column sealed to allow batch reaction for 60 to 90 minutes. Pump tubings were flushed and a sample was taken from the bottom end of the column. Results obtained with this design generally were consistent and more reliable than the earlier modification.

2. Uncontaminated Eglin Soil

Results of batch peroxide stability experiments with uncontaminated soil (Table 25) indicate that the natural peroxide-decomposing capacity of the soil is significantly high. When peroxide solution reacted with soil without any additive (Runs 89-1, 91-2), up to 85 percent of the peroxide decomposed in 90 minutes. Treatment with TMP or TPP only marginally increased the stability (75 percent decomposition in 90 minutes). This slight increase in stability probably was because of the effect of TMP on inorganic catalysts, as will be discussed later in this section.

Adding fluoride, boric acid, or hypophosphite did not increase the stability of peroxide. As noted in Section II, perborate may be an alternative source of peroxide. Perborate salts dissolve in aqueous solutions to

TABLE 25. DECOMPOSITION OF HYDROGEN PEROXIDE IN UNCONTAMINATED EGLIN SOIL.

Run #	Pretreatment Solution*	Pretreatment Time (min)	Peroxide Solution*	Peroxide Concentration (%)	% Peroxide Decomposed 60 min	% Peroxide Decomposed 90 min
64-1	0.001 m TPP	30	TPP + H_2O_2	0.12	79	
68-1	0.001 m TPP		TPP + H_2O_2	0.12	66**	
64-2	0.001 m TMP	30	TMP + H_2O_2	0.12	80	
68-2	0.001 m TMP		TMP + H_2O_2	0.12	65**	
69-1	0.001 m TMP + 5 ppm fluoride	30	TMP + F + H_2O_2	0.12	51**	
73-1	None		TMP + H_2O_2	0.12	25**	
72-1	None		H_2O_2	0.12	29**	
73-2	None		Perborate	0.84	40**	
74-1	0.001 m TMP	30	TMP + perborate	0.84	62**	
77-1	0.001 m TMP + 0.01 m boric	30	TMP + boric + H_2O_2	0.12	89	
78-1	0.03 m perborate + TMP	30	TMP + H_2O_2	0.12		20**
89-1			H_2O_2***	0.12	68	
91-2			H_2O_2	0.06		85
91-3	0.001 m TMP	60	TMP + H_2O_2	0.06		70
92-1	0.001 m TMP + 0.001 m boric	60	TMP + boric + H_2O_2	0.06		75
94-1	0.001 m citric	60	TMP + citric + H_2O_2	0.06		35
98-1	0.0005 m (boric, citric) + 0.001 m TMP	60	boric + citric + TMP + H_2O_2	0.07		75
98-2	10 ppm fluoride + 0.001 m TMP	60	Fluoride + TMP + H_2O_2	0.07		80
100-1	0.015 m perborate + 0.0001 m TMP	60	Perborate + TMP	0.06		44
100-2	0.015 m perborate + 0.0001 m TMP		Perborate + TMP	0.06		72

TABLE 25. DECOMPOSITION OF HYDROGEN PEROXIDE IN UNCONTAMINATED EGLIN SOIL (CONCLUDED).

Run #	Pretreatment Solution*	Pretreatment Time (min)	Peroxide Solution*	Peroxide Concentration (%)	% Peroxide Decomposed 60 min	% Peroxide Decomposed 90 min
120-1	TMP + 0.0005 m (citric, boric)	60	TMP + citric + boric	0.07		82
125-1	0.0001 m citric + 0.001 m TMP	75	Citric + TMP + H_2O_2	0.06		73
125-2	0.0001 m citric + 0.0001 m TMP	75	Citric + TMP + H_2O_2	0.07		80
127-1	0.0001 m catechol + 0.001 m TMP	75	Catechol + TMP + H_2O_2	0.06		79
127-2	0.0001 m catechol + 0.0001 m TMP	75	Catechol + TMP + H_2O_2	0.06		93
130-1	0.001 m hypophosphite + 0.0001 m TMP	60	Hypophosphite + TMP + H_2O_2	0.07		99
130-2	0.001 m hypophosphite + 0.001 TMP	60	Hypophosphite + TMP + H_2O_2	0.07		81
131-1	0.0001 m hypophosphite + 0.0001 m TMP	60	Hypophosphite + TMP + H_2O_2	0.07		85
131-2	0.0001 m hypophosphite + 0.001 m TMP	60	Hypophosphite + TMP + H_2O_2	0.07		85
135-1	0.001 m ascorbic + 0.001 m TMP	60	Ascorbic + TMP + H_2O_2	0.055	82	
135-2	0.002 m ascorbic + 0.001 TMP	60	Ascorbic + TMP + H_2O_2	0.06		83
128-1	0.08% restore	75	Restore + H_2O_2	0.07		86
128-2	0.08% restore	180	Restore + H_2O_2	0.07		86

* Nutrient solution also contains NH_4Cl.
** Solution displaced above the soil due to rapid decomposition; reaction time was less than recorded.
*** Experiments from this row onwards treated with peroxide solution for 60 to 90 min before batching.

produce hydrogen peroxide. The aqueous state of this peroxide may be as free-H_2O_2 or a complex perborate ion, depending upon pH. Several batch experiments were conducted in which the nutrient solutions were spiked with sodium perborate instead of hydrogen peroxide. The stability of perborate-derived peroxide in these experiments did not improve significantly, with an average decomposition of 56 percent in 90 minutes and a range of 44 to 72 percent.

Finally, the effect of three organic additives, ascorbic acid, catechol, and citric acid, on peroxide decomposition was studied. Ascorbic acid (0.001 moles and 0.002 moles) or catechol did not significantly enhance the stability of peroxide. Using different concentrations of TMP with the organic additives also had no effect on peroxide stability. Citric acid in concentrations of 0.001 moles or higher decreased the rate of peroxide decomposition to ~40 percent in 90 minutes. At a lower concentration, (1 to 5 x 10^{-4} moles), citric acid did not increase the stability of peroxide.

Soil samples were taken from the citric acid experiment and plate-counted for total microbial population. This was done to ensure that peroxide stabilization was not achieved at the expense of microbial activity. The microbial population in three soil samples from the citric acid experiment (3.9 to 9.5 x 10^6 CFU/g) was greater than that in natural soil (3 x 10^6 CFU/g). This indicates that the increase in peroxide stability was not detrimental to microbial growth and, therefore, citric acid may be a promising additive for enhanced biodegradation. As noted in Section II, citrate, with no stated purpose, has been used previously in enhanced bioremediation. It is likely that the role of citrate in nutrient formulations is to stabilize hydrogen peroxide.

3. Contaminated Eglin Soil

The rate of hydrogen peroxide decomposition was found to be lower in contaminated soil (Table 26) than in uncontaminated soil (Table 25). Peroxide in untreated, contaminated soil without any additives showed a 45 percent decomposition in 90 minutes. When perborate was used, 60 percent of the peroxide decomposed in 60 minutes. The lower rate of peroxide decomposition

TABLE 26. DECOMPOSITION OF HYDROGEN PEROXIDE IN CONTAMINATED EGLIN SOIL.

Run #	Pretreatment Solution	Pretreatment Time (min)	Peroxide Solution	Peroxide Concentration	% Peroxide Decomposed 60 min	90 min	120 min
75-1	0.001 m TMP	30	TMP + H_2O_2	0.12	61*		
75-2	0.001 m TPP	30	TPP + H_2O_2	0.12			84*
76-1	0.001 m TMP	30	TMP + perborate	0.08			42*
76-2	TMP + 0.03 m perborate	30	TMP + H_2O_2	0.12			25*
77-2	0.01 m H_3BO_3 0.001 m TMP	45	TMP + H_2O_2	0.12			42*
79-1	0.01 m H_3BO_3 0.001 m TMP	30	TMP + perborate	0.08			79*
79-2	0.01 m H_3BO_3 0.001 m TMP	30	TMP + H_2O_2				38
80-1	None		Perborate	0.10	63		
82-2	None		TMP + perborate	0.09	33		
83-1	0.1 m H_3BO_3 + 0.001 m TMP	75	H_2O_2	0.12	1*		
83-2	0.01 m H_3BO_3 + 0.001 m TMP	75	TMP + H_2O_2	0.12	20		
85-1	0.001 m H_3BO_3 + 0.001 m TMP	80	TMP + H_2O_2	0.12	31		
85-2	0.01 m citric + 0.001 m TMP	80	TMP + H_2O_2	0.11	11		
86-1	0.001 m citric + 0.001 m TMP	80	TMP + H_2O_2	0.12	15		
88-1	0.001 m TMP	80	TMP + H_2O_2	0.12		15*	
94-2	--	--	H_2O_2	0.06		45	
95-1	0.001 m TMP	60	TMP + H_2O_2	0.06		45	
95-2	0.001 m H_3BO_3 + 0.001 m TMP	60	H_3BO_3 + TMP + H_2O_2	0.06		45	
96-1	0.001 m citric + 0.001 m TMP	60	Citric + H_2O_2	0.07		17	
96-2	0.0005 m (citric, H_3BO_3) + 0.001 m TMP	60	Citric + H_3BO_3 + TMP + H_2O_2	0.06		8	

in contaminated soil is likely due to the lower microbial populations in these soils (2×10^4 CFU/g) compared to the uncontaminated soils (2×10^6 CFU/g).

Adding TMP or TMP and boric acid to peroxide or perborate solutions did not significantly decrease the rate of decomposition. Citric acid, as in the case of uncontaminated soil, seems to have stabilized peroxide at a concentration of 5×10^{-4} moles or higher. This concentration is slightly lower than needed for uncontaminated soil and may be due to the lower microbial activity in contaminated soil.

4. Abiotic Eglin Soil

Decomposition of hydrogen peroxide was examined in sterilized, uncontaminated soil to estimate the relative contributions of enzymatic and inorganic catalysts. Uncontaminated soil was sterilized by treating with a 1 mg/L $HgCl_2$ solution overnight. This treatment inhibits microbial growth but may not destroy extracellular enzymes. In fact, it may increase the enzymatic activity in the soil by exposing intercellular enzymes upon the destruction of microbes. Therefore, sterilization was achieved also by autoclaving a second sample of soil at 120°C for 30 minutes. Autoclaving deactivates microbial and enzymatic activity, but does not significantly affect the inorganic catalysts, as will be shown later. The destruction of enzymatic activity upon autoclaving was confirmed by using pure catalase as a control.

Results of hydrogen peroxide decomposition in sterilized Eglin soil are given in Table 27. Treatment with $HgCl_2$ decreased the rate of peroxide decomposition in uncontaminated soil to ~60 percent in 60 minutes, compared with 85 percent in untreated soil. Addition of citric acid increased the stability of peroxide significantly (28 percent decomposition in 90 minutes) while TMP did not affect the rate of decomposition. Presence of significant peroxide decomposing capacity in sterilized (mercuric chloride treatment) soils suggest that enzymatic catalysts are dominant in Eglin soil, consistent with the results obtained by Spain et al. (1989). Predominance of enzymatic catalysts in Eglin soils is further confirmed by the slow rate of peroxide decomposition in autoclaved soil (~10 percent in 90 minutes), with or without

TABLE 27. HYDROGEN PEROXIDE DECOMPOSITION IN ABIOTIC EGLIN SOIL.

Run #	Method of Sterilization	Pretreatment		Peroxide Solution	Peroxide Concentration	% Peroxide Decomposed	
		Solution	Time (min)			60 min	90 min
74-2	$HgCl_2$	TMP	30	TMP + H_2O_2	0.121	57	
101-1	$HgCl_2$	TMP	60	TMP + H_2O_2	0.08		59
101-2	$HgCl_2$	TMP + 0.001 m citric	60	TMP + citric + H_2O_2	0.08		28
107-1	Autoclave	--	--	H_2O_2	0.08		15
107-2	Autoclave	10^{-3} m citric + TMP	60	TMP + citric + H_2O_2	0.68		9

any additives. Accordingly, the stabilizing effect of citric acid is due to the inhibition of enzymatic catalysts while that of TMP (in natural soils) is due to the deactivation of inorganic catalysts alone.

5. Homestead Soil

The Homestead soil presented several problems and could not be used with any of the experimental designs described earlier. Therefore, the decomposition of peroxide in Homestead soil was studied by mixing 10 grams of soil with 5 mL solution in an Erlenmeyer flask. The flask was shaken for 30 minutes and a sample was taken at the end of 30 minutes to measure the H_2O_2 concentration.

The decomposition of peroxide in uncontaminated Homestead soil was very rapid, 90 percent in 30 minutes. Adding TMP or citric acid did not decrease the rate of decomposition.

Upon autoclaving the Homestead soil, peroxide decomposed at a rate of -50 percent in 30 minutes. This indicates that nonenzymatic catalysts play a relatively major role in decomposing peroxide in Homestead soil. Further, these catalysts do not seem to be deactivated by TMP or citric acid, either because of the nature of the catalysts or because TMP/citrate were removed from the solution by sorption on the soil.

6. Aqueous Solutions

Experiments with sterilized Eglin and Homestead soils indicate different mechanisms of peroxide decomposition in the two soils. In addition, the effect of additives also seems to vary depending upon the nature of the catalysts or soil. To clarify the role of inorganic catalysts and their inhibition by additives, several experiments were conducted with Fe-enriched solutions. In these experiments $FeSO_4$ or $FeCl_3$ solution were prepared using deionized water and the pH of the solutions was adjusted near 7. Approximately 100 mL of aqueous Fe solutions were spiked with the additives and H_2O_2 was added to the 100 mL aliquot. The concentration of H_2O_2 was monitored over 24 hours. The effect of heating during sterilization on the inorganic catalysts of peroxide decomposition in the soil was evaluated by autoclaving the Fe solutions before spiking with H_2O_2 and other additives.

Results of the aqueous batch experiments are given in Table 28. Nearly complete decomposition of peroxide occurred within 12 hours in solutions with no additives. Sodium phosphate, TMP, and citric acid inhibited peroxide decomposition to varying extents. Differences in the degree of inhibition of the three additives can be attributed to the concentration of the additive and to the extent of Fe-complexation by the additive.

Decomposition of peroxide decreased to ~25 percent over 24 hours in autoclaved solutions. The increased stability of peroxide may be due to the deactivation of inorganic catalysts by heating. Alternatively, the precipitation of Fe-oxide/hydroxide during heating may have removed most of the iron from solution and, therefore, prevented the reaction with peroxide. Analysis of a filtered (0.22 μm) sample indicated <1 mg/L dissolved Fe, compared with 36 mg/L in the stock solution. A new $FeSO_4$ solution was therefore prepared and a precipitate was formed by increasing the pH to ~10. The solution was thoroughly mixed to maximize precipitation. The pH was adjusted slowly to ~7 and H_2O_2 (0.14 percent) was added. The stability of peroxide was similar in this experiment as in the autoclaved $FeSO_4$ solution. Analysis of dissolved Fe concentration again indicated <1 mg/L Fe.

Results of experiments using autoclaved solutions suggest that peroxide decomposition is catalyzed by Fe in the ionic form with only a minor contribution from Fe present as suspended solids. Consequently, autoclaving may not affect the activity of inorganic catalysts of peroxide decomposition in the soils. The extent of complexation of ionic Fe by the additive determines the degree of increased peroxide stabilization.

C. NONBIOLOGICAL OXYGEN DEMAND

Both organic and inorganic components of soils react with oxygen upon injection of oxygen-enriched solutions. Oxygen-consuming reactions with the organic components include growth of microbial populations and, to a lesser extent, chemical oxidation of hydrocarbon or soil-organic matter. These are the desirable reactions for a biodegradation operation. However, oxygen is consumed also by inorganic reactions involving the oxidation of multivalent elements, such as iron (Fe), manganese (Mn), sulfur (S), carbon (C) and

TABLE 28. DECOMPOSITION OF HYDROGEN PEROXIDE IN AQUEOUS FE SOLUTIONS.

Run #	Fe Concentration (mg/l) Total	Fe Concentration (mg/l) Dissolved*	Fe-Salt	Autoclaved	Additive	Peroxide Conc. (%)	% Decomposed 12 hr	% Decomposed 24 hr
1	57	~52	$FeSO_4$	No	--	1.1	81	~100
2	36	<1	$FeSO_4$	No	--	0.14	26	27
3	36	<1	$FeSO_4$	Yes	--	0.14	25	25
4	32	21	$FeCl_3$	No	--	0.18	78	86
5	36	<1	$FeCl_3$	Yes	--	0.18	25	25
6	57	~52	$FeSO_4$	No	10^{-2} m Na_3PO_4	1.2	25	27
7	57	~52	$FeSO_4$	No	10^{-3} m TMP	1.3	60	70
8	57	~52	$FeSO_4$	No	10^{-3} m citric acid	1.2	73	85
9	36	<1	$FeSO_4$	Yes	10^{-3} m Na_3PO_4	0.18	25	25
10	36	30	$FeSO_4$	No	10^{-3} m Na_3PO_4	0.11	38	39

* Concentration in solution after filtration through 0.22-μm membrane filter.

nitrogen (N). Oxygen consumption by the inorganic reactions should be estimated to determine the total oxygen demand of the system.

1. Experimental Studies

Batch experiments were conducted to estimate the nonbiological oxygen demand. Columns filled with contaminated, natural or autoclaved Eglin soil were eluted with two to three pore-volumes of oxygen-enriched deionized water. The columns were then sealed to allow soil-solution reaction for 1 to 20 hours. At the end of the experiment, dissolved oxygen (D.O.) concentration in the solution was measured using the CHEMETRICS™ kit.

Data for the consumption of oxygen in batch experiments (Table 29) show that oxygen consumption in both the natural and autoclaved soils was significant in the first 1 or 2 hours of reaction.

TABLE 29. OXYGEN DEMAND IN NATURAL AND AUTOCLAVED CONTAMINATED EGLIN SOIL.

Time (hours)		Natural Soil D.O. (mg/L)		Autoclaved Soil D.O. (mg/L)	
Total	Reaction	Initial	Final	Initial	Final
1	1	16.0	5.5	17.0	11.0
2	1	15.0	6.0		
21	20			16.0	10.0
22	20	16.0	1.0		
24	2	14.0	11.0	7.0	7.0

Oxygen demand in the autoclaved soil was nearly exhausted within ~20 hours and oxygen was not consumed during a two hour experiment conducted immediately after the 20-hour experiment. In contrast, oxygen demand in the natural soil was significant even after ~24 hour reaction because of biological activity.

2. Discussion

Experimental data presented above indicate that nonbiological reactions play only a minor role in the consumption of oxygen in biologically active soils. Further, the inorganic reactions are self-inhibited upon continued interaction with oxygen-enriched solutions. Oxidation of aqueous species of several multivalent elements, Fe, Mn, S, C, and N, may be responsible for the decrease in D.O. concentration in autoclaved soils. However, oxidation of aqueous Fe frequently is the most important for inorganic oxygen demand in shallow soil environments. Concentration of Mn in soils generally is much lower than that of Fe and nonbiological oxidation of the species of S, C and N is kinetically inhibited at low temperatures (Stumm and Morgan, 1981).

The kinetics of aqueous Fe oxidation is given by the equation:

$$\frac{d\,(Fe^{2+})}{dt} = -K\,[Fe^{2+}]\,PO_2[OH^-]^2 \qquad \text{(E-11)}$$

where PO_2 is the partial pressure of oxygen, and K is the rate constant; square brackets represent the aqueous concentration of a species. Based on a rate constant of 2×10^{13} M^{-2} atm^{-1} min^{-1} (Davison and Seed, 1983), the half-life of Fe oxidation at a pH of 7 is ~ 1 minute in oxygen-saturated (40 mg/L) solutions and ~10 minutes in solutions with 20 mg/L D.O. The aqueous oxidation of Fe^{2+} can be represented as:

$$Fe^{2+} + 1/4\ O_2(aq) + H^+ = Fe^{3+} + 1/2\ H_2O \qquad \text{(R-20)}$$

Thus, 0.25 moles of oxygen are consumed for each mole of iron oxidized. As suggested by soil chemical composition (Table 12), inorganic oxygen demand in experiments with the Eglin soil was likely due to the oxidation of Fe. Total Fe oxidized during the experiments can be estimated using data in Table 29 and the stoichiometry of the oxidation reaction (Reaction R-20).

The cumulative oxygen consumption in experiments with the autoclaved soil was ~12 mg/L. In addition, some oxygen must have been consumed during initial elution with oxygen-enriched solutions prior to batch reactions. Thus a conservative estimate of the total oxygen consumed may be ~15 mg/L. The soil mass in the column was 220 grams with ~50 mL solution.

A decrease in D.O. concentration by 15 mg/L from a 50 mL quantity of solution represents a loss of 2.3×10^{-5} moles of oxygen. According to

Reaction (R-20), this amount of oxygen would have oxidized 9.4×10^{-5} moles or 5.3 mg of Fe, corresponding to 24 mg Fe / Kg soil. Thus, less than 10 percent of the total Fe in the soil (357 mg/L, Table 12) may have been oxidized in the experiments described above.

Because there is no aqueous Fe in the influent solutions, Fe must have been leached from the soil. A major portion of the Fe in contaminated soil probably is present in the minor quantities of clay minerals in the soil. The oxidation of Fe in the experiments may have proceeded in a two-step process. The first step would be the dissolution of a Fe^{2+}-bearing phase or the adsorption of aqueous O_2 on a surface Fe^{2+} site. The second step would be the oxidation of Fe^{2+}. Oxidation of reduced iron present in the clay minerals or other phases would eventually lead to the precipitation of a ferric hydroxide layer which may inhibit further oxidation and may lower the oxygen consumption rates.

The above analysis of possible mechanisms of nonbiological oxygen consumption in the Eglin soil is consistent with the results of the batch experiments. As noted earlier, the oxygen demand in the autoclaved soils was very little and decreased after continued reaction for ~20 hours. This suggests that nonbiological oxygen demand in field operations will be minimal and will decrease with time. However, if the redox state of the system decreases, for example during system shutdown period, the nonbiological oxygen-consuming capacity of the soil may be regenerated.

D. EXPERIMENTS WITH AQUIFER SIMULATOR

Aquifer simulator experiments were conducted to study on a larger scale the expected performance of polyphosphates and a peroxide stabilizer in the field. Another objective was to characterize geochemical processes that may occur in the aquifer during enhanced biodegradation operations.

1. Design

The simulator was constructed from a glass tank measuring 72 inches by 30 inches by 7 inches. Infiltration and production galleries (~12 inches each) were created by placing gravel to a height of 10 inches on the two ends

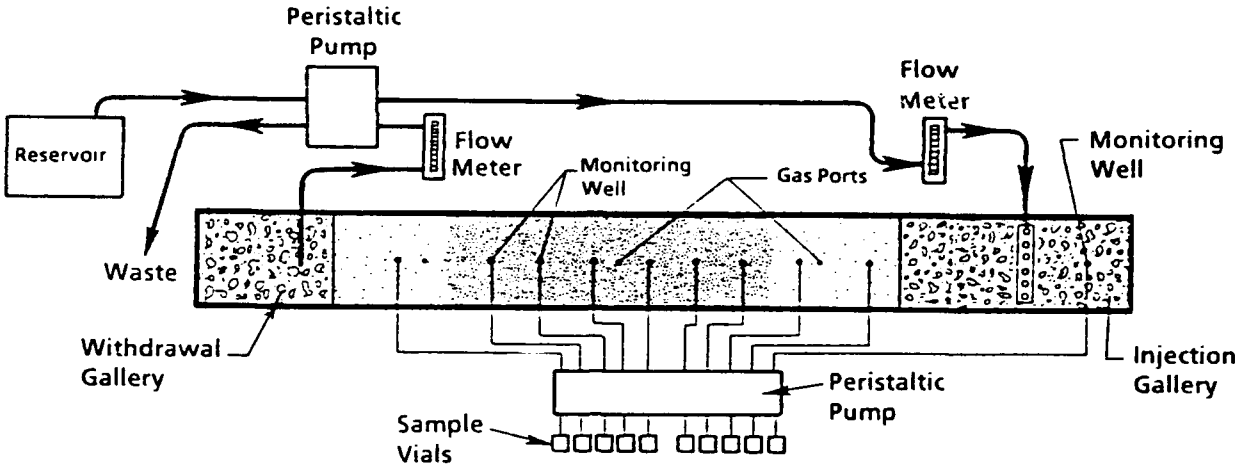
of the tank (Figures 3 and 4). The ~48-inch space between the gravel zones was filled with zones of uncontaminated soil sandwiching a zone of contaminated soil. The soil was saturated to a height of ~8 inches and the tank was covered with a Plexiglas lid. Both the uncontaminated and contaminated soils were obtained from the Eglin AFB. Nutrient solutions were injected into the injection gallery using a peristaltic pump and the injection rate was monitored using a flow meter. A similar setup was used for pumping solutions from the production gallery. Baffles were placed in both the injection and production galleries to avoid channeling.

Nine monitoring wells were placed in the soil zone and one in the infiltration gallery. The wells were constructed by inserting a 0.25-inch diameter stainless steel tubing in the soil or gravel. A stainless steel screen was spot welded on the lower end of the tube to avoid the flow of wet soil into the well. Teflon tubing [1/8-inch internal diameter (i.d.)] was placed into the well connected to a peristaltic pump for drawing samples. The wells were numbered in a reverse order. Well 10 was in the input gallery. In the soil zone, the well closest to the input gallery was numbered 9 and the one closest to the output gallery was numbered 1. The locations of the various wells in the soil zone with respect to the input gallery are noted in Appendix C. In addition to the monitoring wells, four gas sampling ports were installed in the unsaturated zone by inserting a 1/8-inch steel tubing.

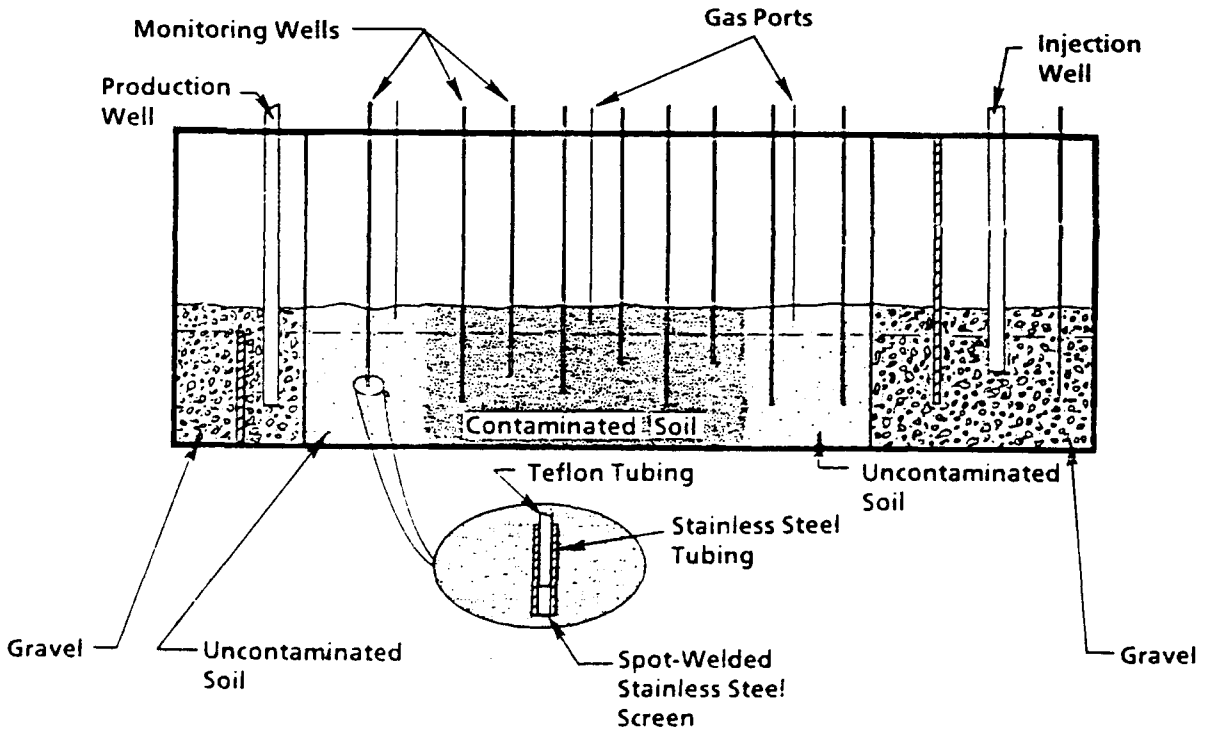
2. Hydraulic Parameters

The tank was raised by 3 inches on the input side to provide a natural gradient. The input and output pumping rates were set at ~25 ml/minute, corresponding to a velocity of about 1 meter/day and hydraulic conductivity if 2.2×10^{-2} centimeters/second (cm/sec). The water level in the aquifer was constantly monitored to ensure that the constant inflow and outflow rates were a true indication of the flow rate in the aquifer.

A tracer test was conducted using an NaCl solution to verify the flow rates in the aquifer simulator. Concentration of chloride was measured in the monitoring wells at 15-minute intervals. The test was run for 4 hours and the



TOP VIEW



SIDE VIEW

Figure 3. Two Views of the Aquifer Simulator. Monitoring Wells are Numbered Consecutively with Well 10 Near the Injection Well and Well 1 Near the Production Well.

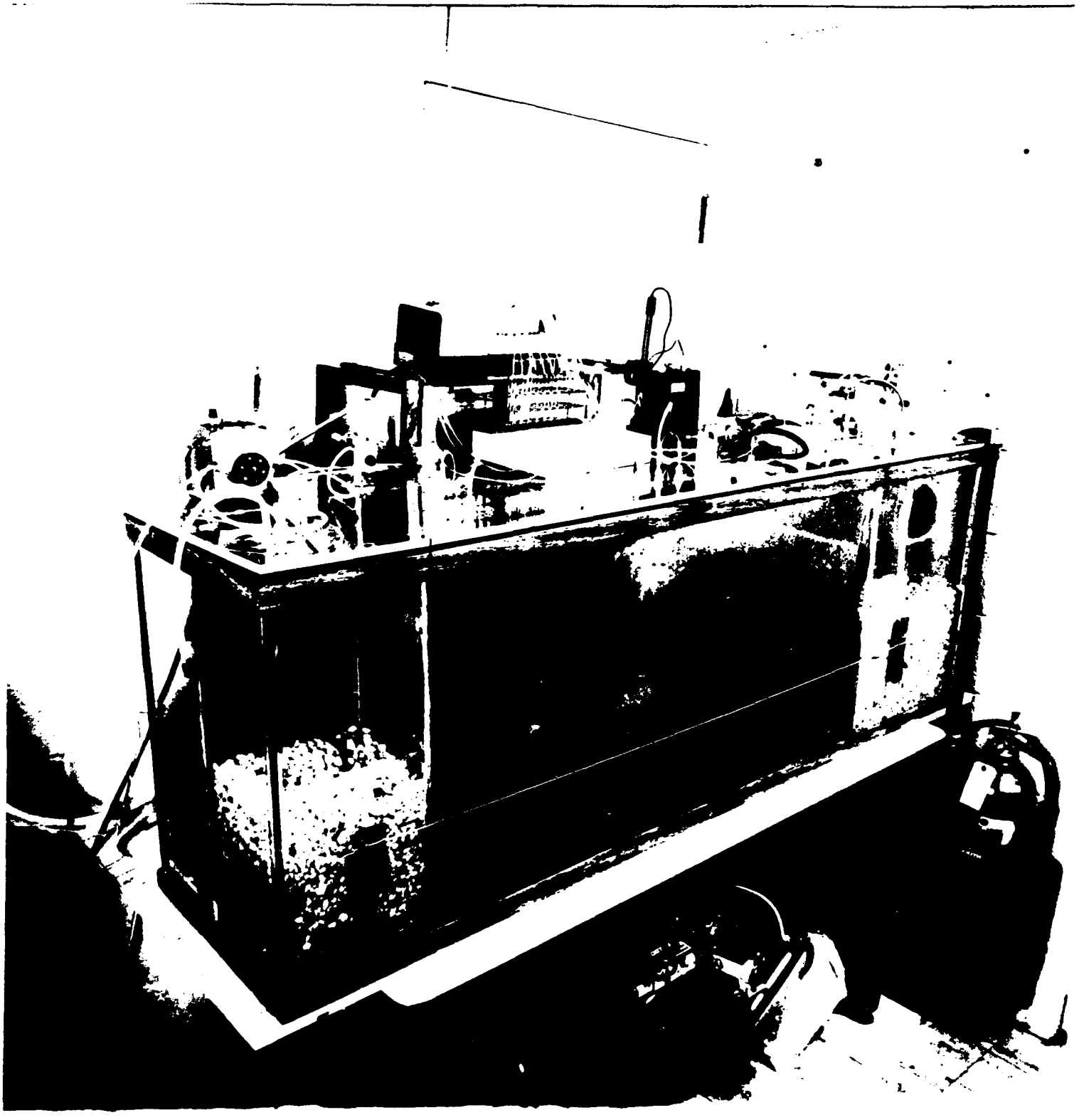


Figure 4. A Picture of the Laboratory Setup Used for the Aquifer Simulator Experiments.

data were modeled using a one-dimensional solute transport computer program ODAST (Javandel et al., 1984). The tracer test data are reproduced reasonably well using a value of 0.08 for the dispersion coefficient and 1.25 meters/day for velocity (Figure 5). These values of dispersion coefficient and velocity are similar to those found in unconsolidated sandy aquifers (Freeze and Cherry, 1979).

3. Results

Nutrient solutions in the aquifer simulator experiments consisted of various proportions of TMP and citric acid along with other additives (Table 30).

TABLE 30. INPUT COMPOSITIONS OF NUTRIENT SOLUTIONS USED IN THE AQUIFER SIMULATOR EXPERIMENTS.

Time (hours)	Solution Composition* TMP (mg/L)	Citric Acid (mg/L)	Na3PO4 (mg/L)	Peroxide (%)
0	306	206		
52	300	206		0.09%
59	97	67	28	
123	97	67	28	0.065
145	97	206	28	0.065
197**	97	--	28	0.065
218	injection of nutrients stopped for 50 hours			
291	306	206	28	0.12
323	0.08% RESTORE 375 Formulation		0.065	

* Other additives (mg/L): KCl = 20; NH_4Cl = 50

** Instead of Citric Acid, Ascorbic Acid (352 mg/L) and $CUSO_4.5H_2O$ (2.5 mg/L) were added as peroxide stabilizers

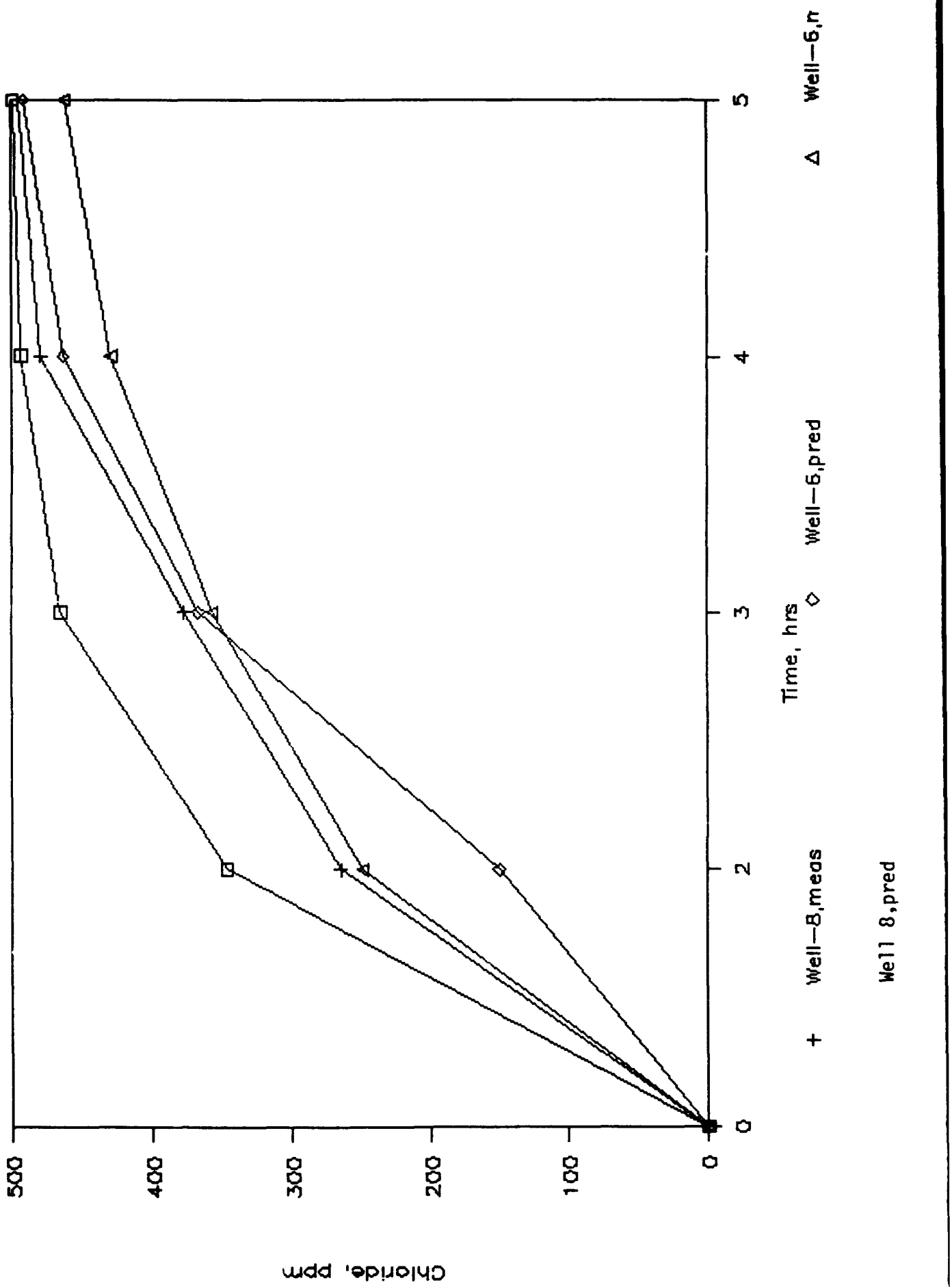


Figure 5. Chloride Transport (Predicted and Measured).

These solutions were injected continuously for ~450 hours, with an ~50-hour break after 218 hours. Initially, to evaluate the performance of TMP, samples from all the monitoring wells were analyzed for orthophosphate. Selected samples were analyzed also for total P, Ca, Mg, Na, K, Fe, and Mn concentrations using an inductively coupled plasma spectrophotometer (ICP). Once the hydrolysis of TMP was characterized, analysis of phosphate and other elements was performed less frequently.

The transport of hydrogen peroxide was monitored by measuring the peroxide concentration in samples from the injection gallery (Well 10) and in two wells closest to this gallery (Wells 8 and 9). Results of the experiments are described below.

a. Performance of TMP

The ortho and total phosphate concentrations in two of the monitoring wells, Wells 1 and 9, are shown in Figures 6 through 14. Input solutions for the first 59 hours contained <3 mg/L of orthophosphate. An increase in orthophosphate concentration, due to the hydrolysis of TMP, occurred nearly one foot into the soil zone. To increase the availability of orthophosphate for microbial growth before significant hydrolysis of TMP occurred, ~20 mg/L orthophosphate were added to the input solutions used after 59 hours. As the solutions moved through the soil zone, the orthophosphate content first decreased slightly, probably due to sorption (adsorption and precipitation). Continuing hydrolysis of TMP increased orthophosphate relative to polyphosphate, but sorption and/or microbial growth decreased the total-P content.

The behavior of the ortho and polyphosphate species in the aquifer simulator was modeled using the ODAST solute transport code. The code was modified to include an algorithm for TMP hydrolysis (Appendix C). The transport parameters were based on the Cl^- trace test and the hydrolysis parameters were varied until a close match was obtained between calculated and observed values.

Agreement between the measured and predicted values at 4.25, 5.5, and 22.3 hours is fairly good for the first three wells (Figure 15). However,

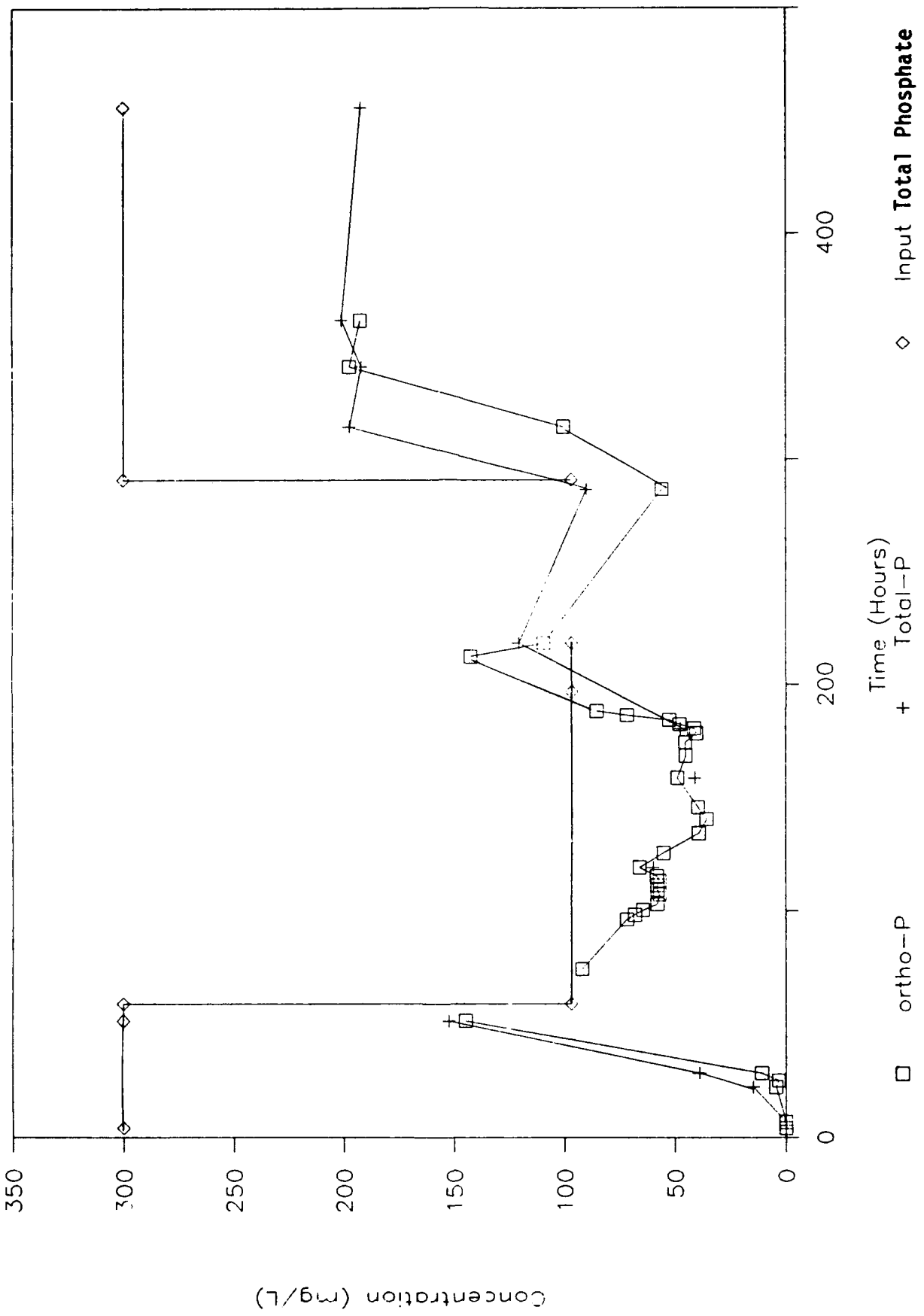


Figure 6. Phosphate Concentrations in Well 1.

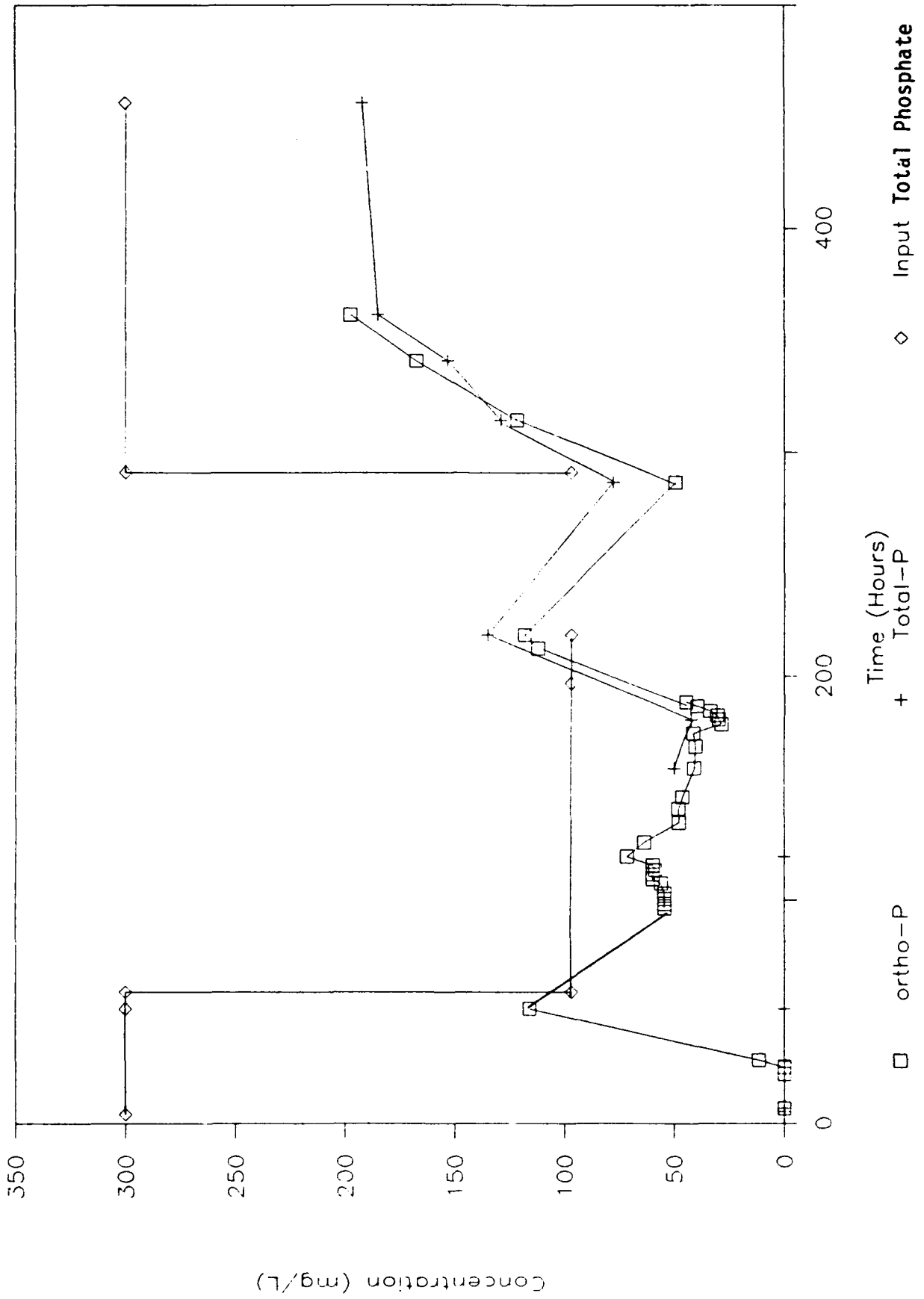


Figure 7. Phosphate Concentrations in Well 2.

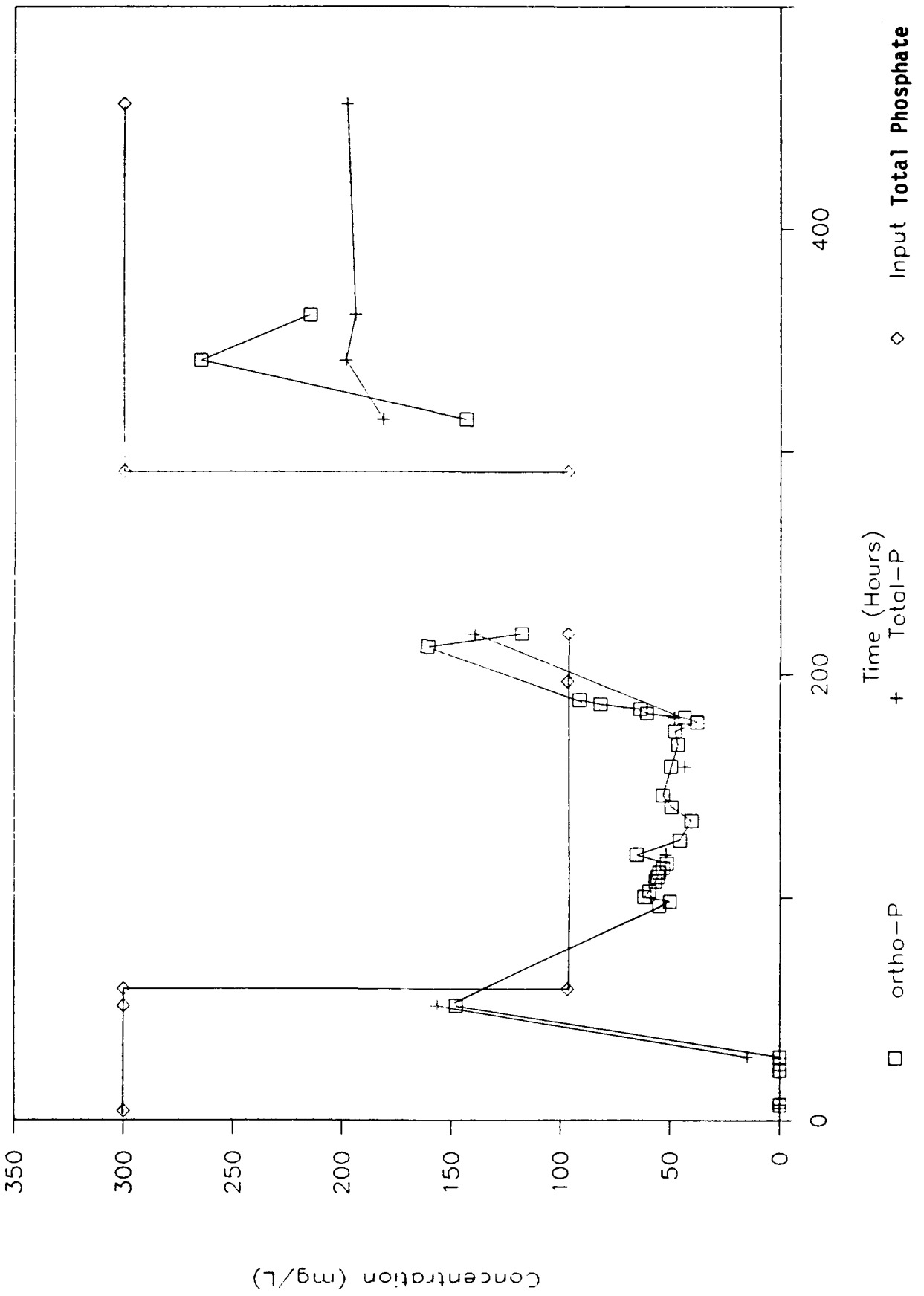


Figure 8. Phosphate Concentrations in Well 3.

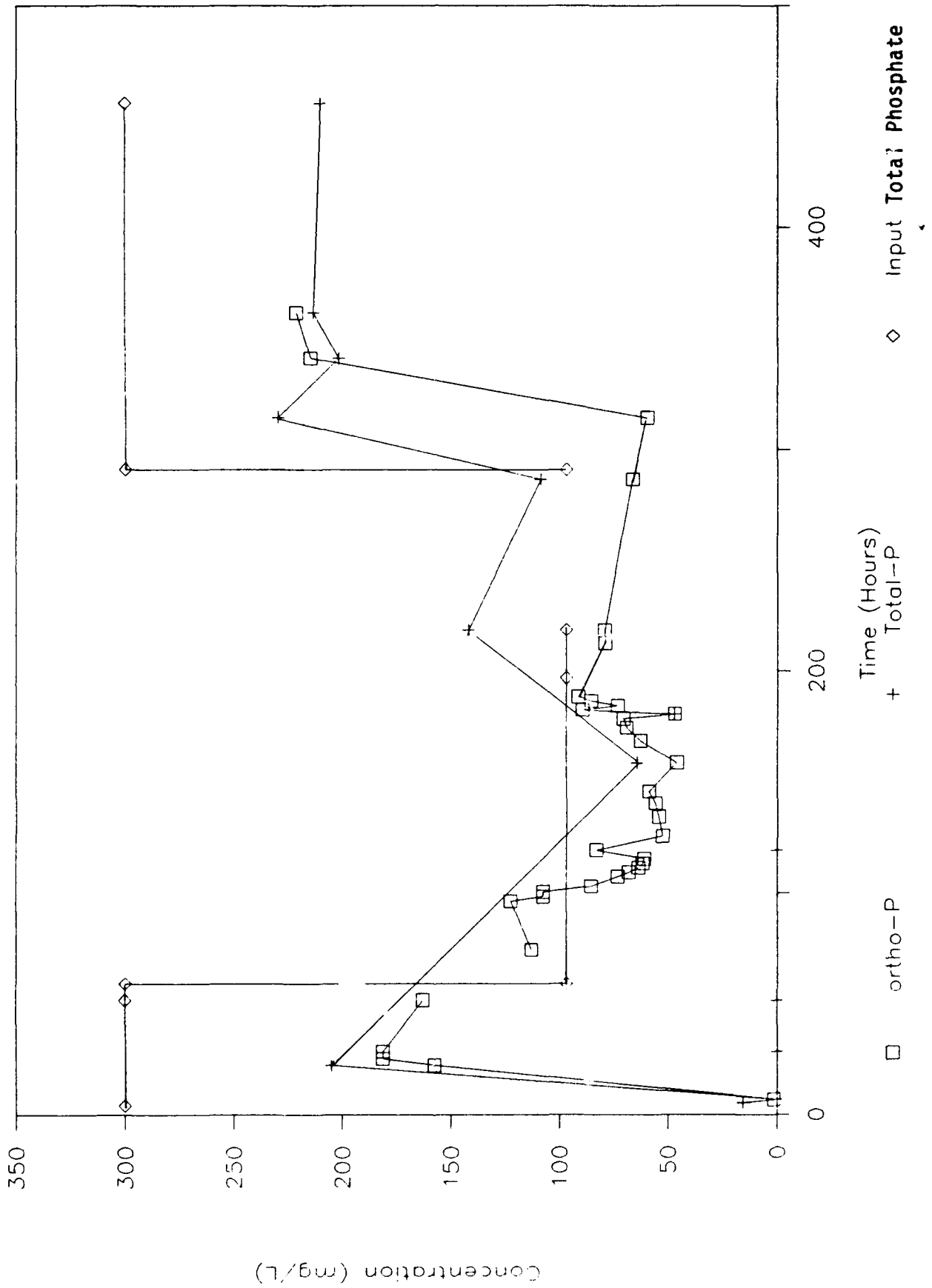


Figure 9. Phosphate Concentrations in Well 4.

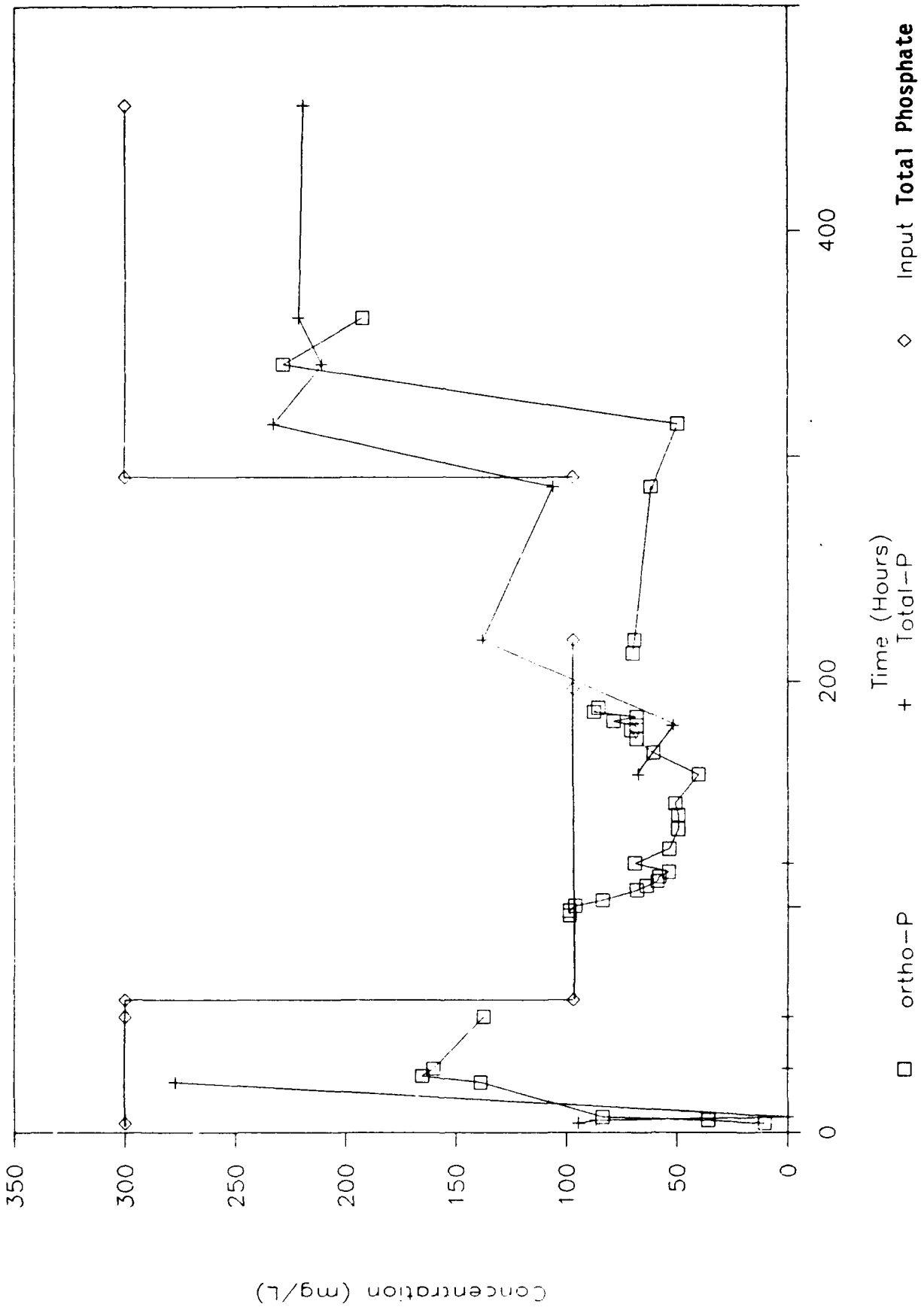


Figure 10. Phosphate Concentrations in Well 6.

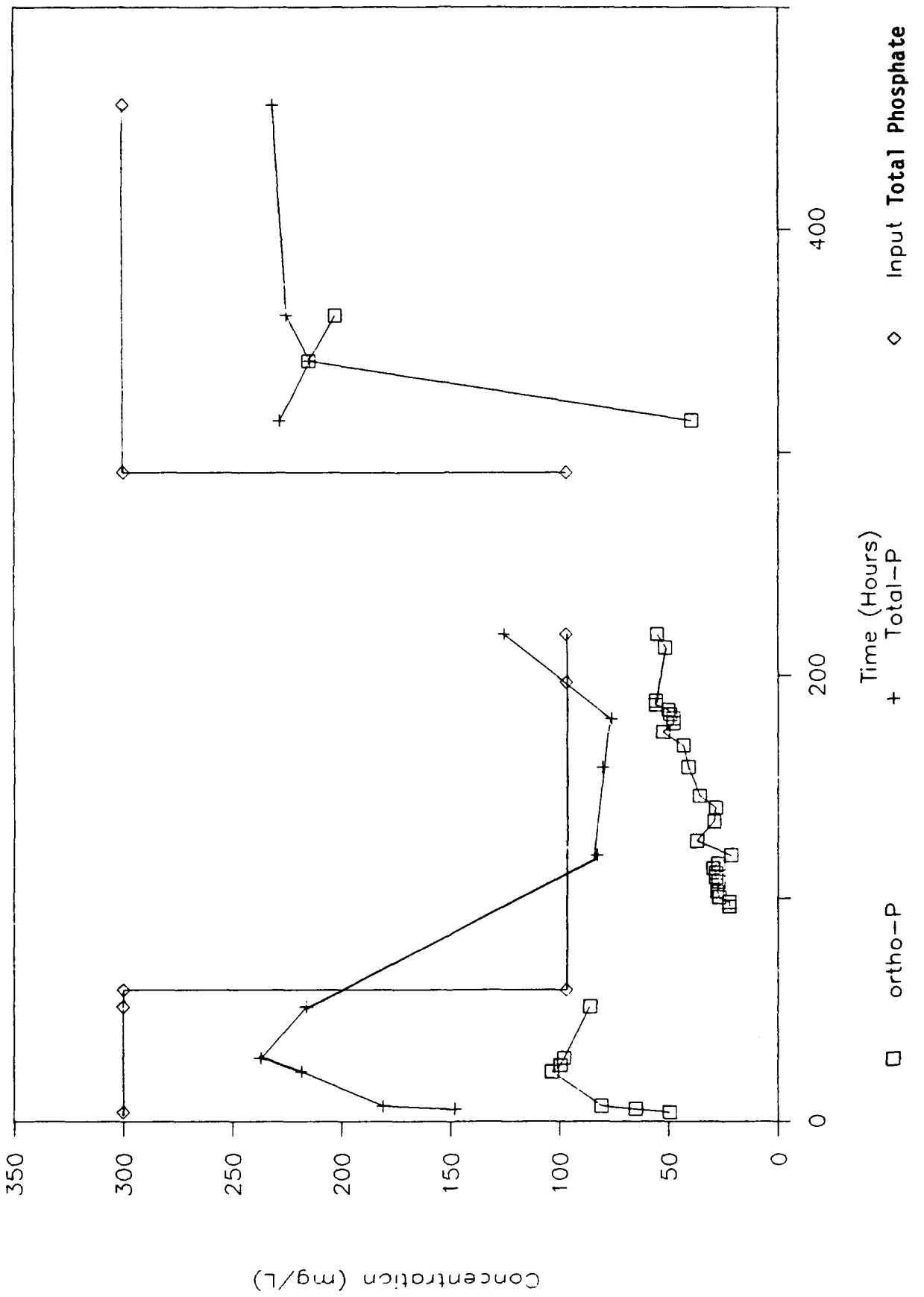


Figure 11. Phosphate Concentrations in Well 7.

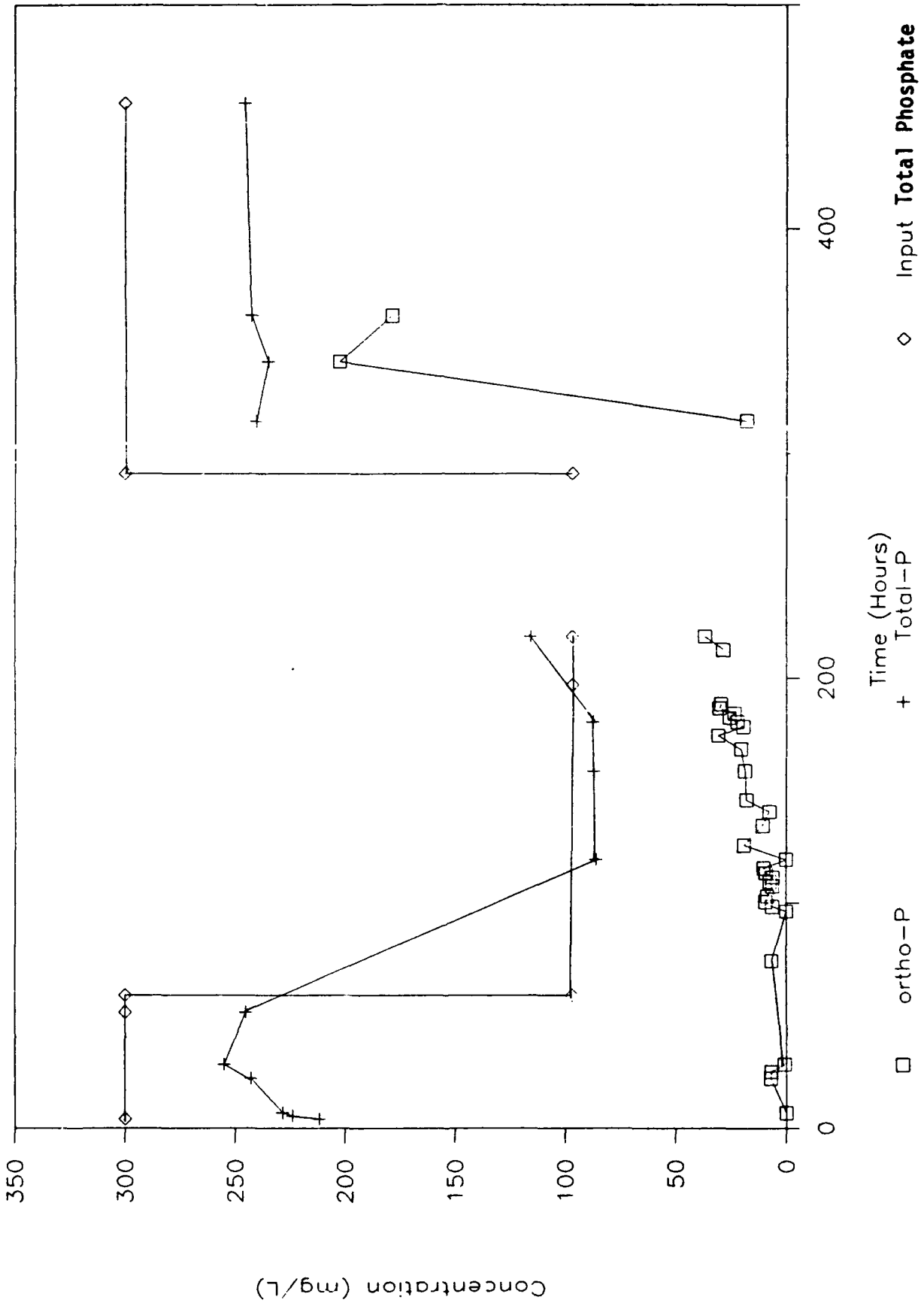


Figure 12. Phosphate Concentrations in Well 8.

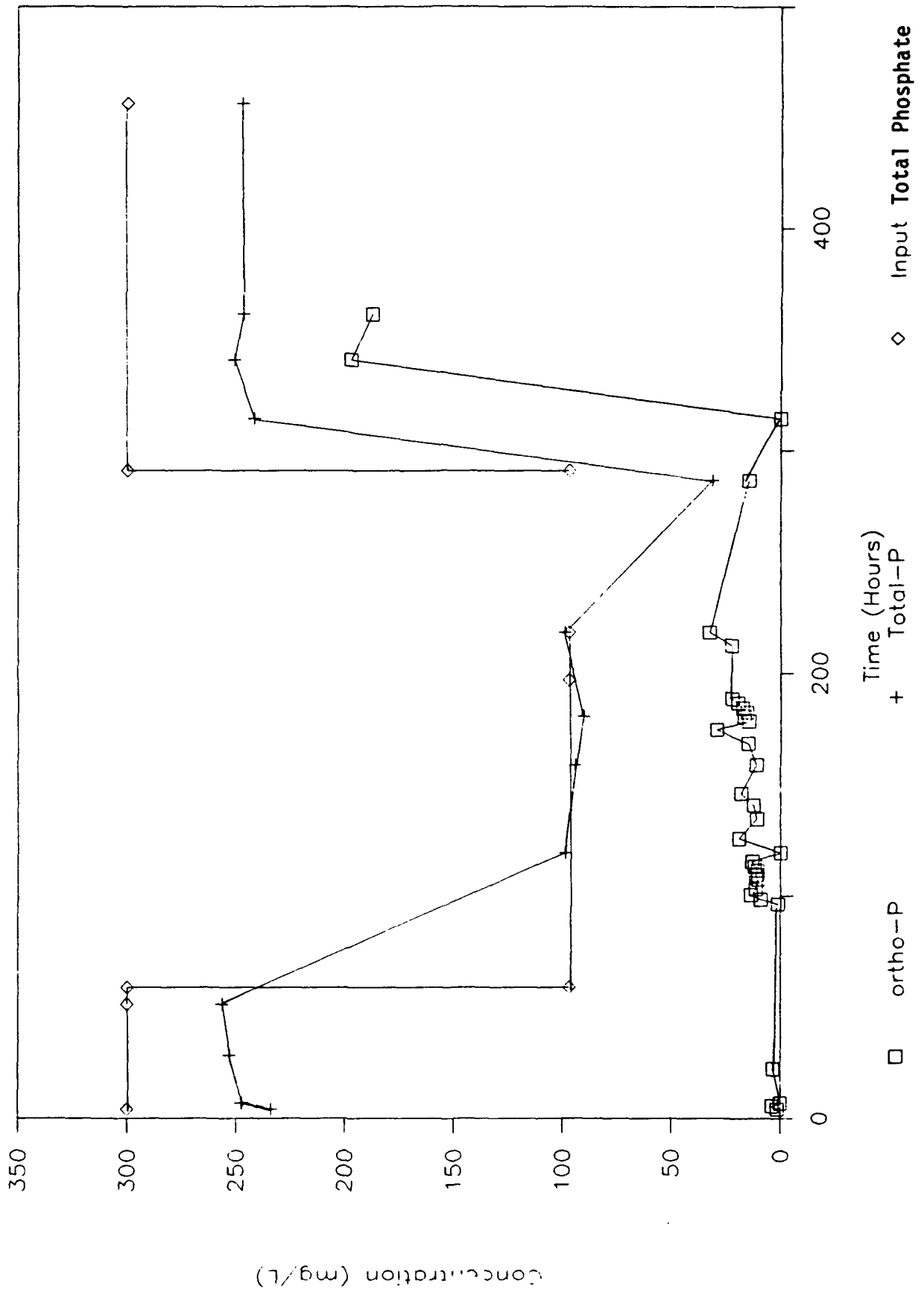


Figure 13. Phosphate Concentrations in Well 9.

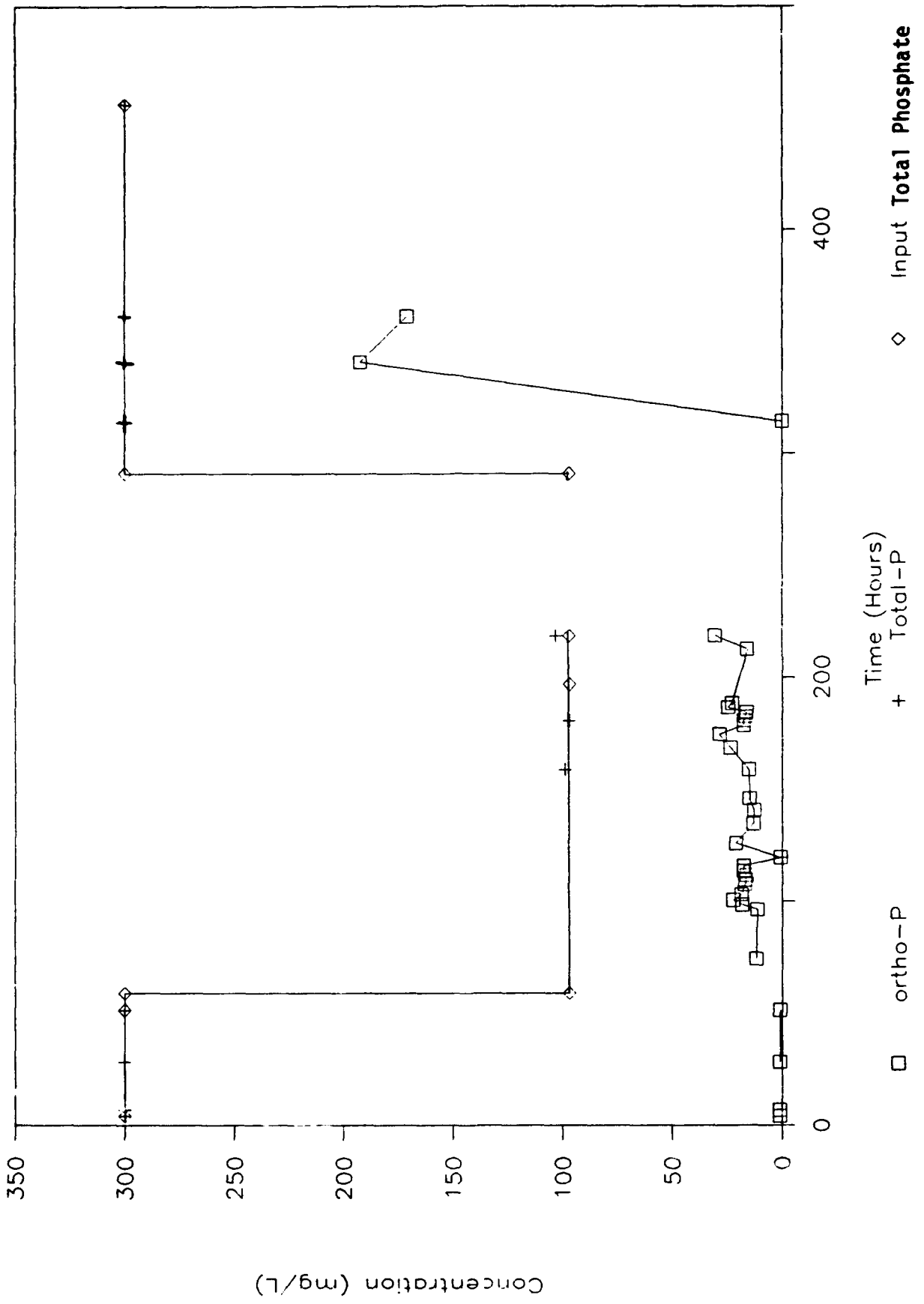


Figure 14. Phosphate Concentrations in Well 10.

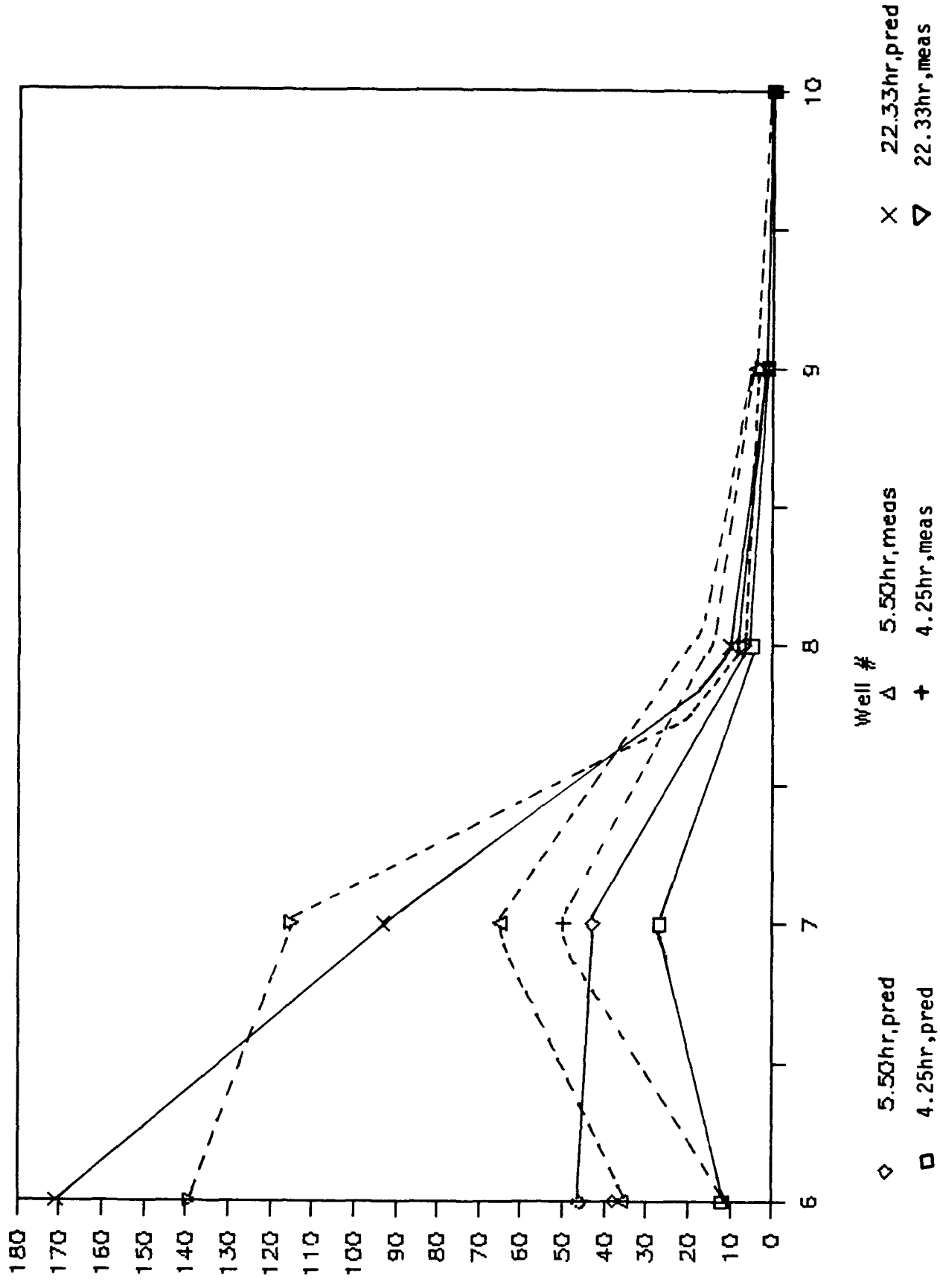


Figure 15. Ortho-Phosphate Concentrations (Predicted and Measured).

the predicted concentrations are significantly higher than those measured in wells ~12 inches and further into the soil zone. This disagreement is probably because of the lack of a retardation term in the model. Using a value of ~1.5 for the retardation factor produces a better fit with the measured values. However, in order to produce agreement with measured values from all the wells, the retardation constant needs to be adjusted at least at two places. This is because adsorption probably is the dominant sorption process in the early part of the soil zone. Continued hydrolysis of TMP results in the precipitation of a phosphate phase which then controls the dissolved orthophosphate concentration and the consequent retardation factor may be different than that due to adsorption.

Hydrolysis constants for TMP used to produce the values in Figure 15 suggest a lower rate of hydrolysis compared with that based on column studies. For the uncontaminated zone, rate constants are very similar, lower only by a factor of 2, but for the contaminated zone, the difference is >1 order of magnitude. Because the column experiments were conducted with a short residence time (~1 hour), the present values are more reliable. In addition, the values based on the aquifer simulator data are in better agreement with values from the literature (Dick and Tabatabai, 1986).

Although retardation of phosphates occurred in the aquifer simulator experiments, a large-scale precipitation is not apparent from the experimental data as water velocity in the aquifer did not decrease during the experiment. Data for the concentration of Ca also are consistent with minimal precipitation. As can be seen in Figures D-1 through D-9 (Appendix D), the Ca concentration decreased from input values of ~30 mg/L to ~15 mg/L towards the output gallery. However, measured Ca values near the output gallery were lower in the beginning (50 to 158 hours) compared with values in the later part of the experiment. The lower Ca concentrations probably reflect greater precipitation of Ca phosphates early in the experiment because of a higher concentration of TMP (total P = ~280 mg/L as PO_4) in the input solutions from 0 to 54 hours. Hydrolysis of TMP in these solutions will produce a higher concentration of orthophosphate, resulting in

a higher degree of supersaturation and, therefore, a lower kinetic barrier to phosphate mineral precipitation.

b. Stability of Hydrogen Peroxide

Hydrogen peroxide was added to the input solutions in the aquifer simulator experiment. The effect of pretreatment with nutrient solutions containing stabilizing additives as well as the simultaneous addition of peroxide and stabilizing additive was evaluated. Citric acid was the primary additive tested in these experiments. The effect of ascorbic acid on peroxide stability also was evaluated. In addition, the RESTORE 375™ formulation was tested to confirm the results obtained from the field demonstration at the Eglin AFB. Compositions of the additives in the nutrient solutions are given in Table 30.

The concentration of peroxide initially was measured in all the monitoring wells. However, it became apparent that peroxide was not stable in the soil zone. Consequently, measurements were restricted to the two wells closest to the input gallery until peroxide was detected in one of the two wells.

Results of peroxide stability experiments are given in Table 31. Peroxide was almost completely stable over an extended period of time in the input gallery. However, peroxide was never detected in the first monitoring well (Well 9) located only ~2.5 inches into the soil zone, corresponding to a travel time of ~1.5 hours. Rapid decomposition of hydrogen peroxide in Eglin soil is consistent with field observations at the Eglin AFB (EAES, 1989; Spain et al., 1989). Note that greater stability of peroxide was expected on the basis of batch experiments conducted earlier. This apparent discrepancy between the batch and flow-through experiments is likely due to gas formation and incomplete reaction in the batch experiments. Because of the possibility of an incomplete reaction in batch experiments, the results of this study are not directly comparable to those of Lawes (1988).

Monitoring of D.O. in the contaminated zone indicated that oxygen was consumed rapidly after contact with the contaminated soil. This is consistent with measured Fe concentrations (Figures D-1 to D-9, Appendix D)

TABLE 31. HYDROGEN PEROXIDE CONCENTRATION (%) IN THE INPUT GALLERY (WELL 10) AND TWO WELLS IN THE SOIL ZONE DURING THE AQUIFER SIMULATOR EXPERIMENT.

Time (Hours)	Wells 8	9	10
123.50	0.008	0.008	0.038
124.25	0.004	0.008	0.019
125.50	0.000	0.000	0.025
127.50	0.008	0.010	0.025
130.75	0.006	0.006	0.047
131.75	0.004	0.006	0.042
132.75	0.003	0.004	0.048
133.75	0.004	0.004	0.050
134.75	0.003	0.006	0.053
135.75	0.003	0.005	0.053
136.75	0.004	0.004	0.055
137.75	0.004	0.004	0.055
139.75	0.004	0.004	0.056
142.75	0.008	0.008	0.055
148.25	0.008	0.008	0.057
149.25	0.008	0.008	0.057
151.00	0.008	0.008	0.059
155.25	0.008	0.008	0.059
157.25	0.006	0.006	0.070
159.25	0.006	0.008	0.067
161.25	0.008	0.010	0.070
163.25	0.008	0.034	0.067
166.50	0.008	0.008	0.060
168.25	0.006	0.008	0.066
172.25	0.004	0.006	0.057
174.25	0.006	0.006	0.065
193.00	ERR	0.004	0.056
193.50	0.000	0.020	0.056
194.25	0.000	0.017	0.060
195.00	0.000	0.017	0.060
195.50	0.000	0.008	0.056
197.25	0.008	0.008	0.042
197.50	0.008	0.008	0.050
197.75	0.008	0.008	0.053
198.25	0.008	0.008	0.046
204.00	0.004	0.004	0.047
206.00	0.004	0.004	0.049
208.00	0.004	0.010	0.057
210.00	0.008	0.008	0.057
212.00	0.008	0.008	0.057
214.00	0.006	0.006	0.053
216.00	0.006	0.004	0.053

TABLE 31. HYDROGEN PEROXIDE CONCENTRATION (%) IN THE INPUT GALLERY (WELL 10) AND TWO WELLS IN THE SOIL ZONE DURING THE AQUIFER SIMULATOR EXPERIMENT (CONCLUDED).

Time (Hours)	Wells 8	9	10
218.00	0.006	0.004	0.054
293.50	0.004	0.006	0.117
299.50	0.013	0.011	0.105
305.50	0.006	0.013	0.105
310.25	0.015	0.011	0.113
314.00	0.006	0.010	0.103
317.25	0.009	0.010	0.103
323.00	0.010	0.008	0.061
329.50	0.008	0.013	0.067
333.50	0.006	0.010	0.065
340.75	0.016	0.010	0.065
346.00	0.008	0.010	0.057
357.00	0.006	0.006	0.067
361.00	0.010	0.008	0.065
365.00	0.010	0.010	0.057

which indicate higher levels soon after the point where contaminated soil is encountered (Well 8). The solubility of reduced iron is much greater than that of oxidized iron and the changes in iron concentration reflect a change in the redox state of the system.

Note that Fe concentrations in the initial samples from nearly all wells were much higher, reaching up to ~30 mg/L. This is because pore water prior to the beginning of the experiment equilibrated with the soil under increasingly reduced conditions resulting in considerable dissolution of iron from the soil.

Concentrations of CO_2 and O_2 in the gas ports were also measured. CO_2 concentrations reached six percent immediately above the contaminated soil and ~0.3 percent in the uncontaminated soil. Consequently, the consumption of oxygen in the contaminated soil was probably due to microbial growth. Oxygen concentrations in the headspace above the soil zone ranged from 19.7 percent to 21 percent. This variation is not very significant because the measurement error was nearly $\pm$ 1 percent. Although hydrogen peroxide decomposed immediately upon entering the soil zone, significantly high O_2 concentrations were not measured. This is because higher O_2 levels may have been masked partly by homogenization and partly by O_2 consumption in the uncontaminated zone.

SECTION V

DIAGNOSTIC PROCEDURES

As noted earlier in this report, existing nutrient formulations for enhanced biodegradation generally lead to plugging due to excessive precipitation. The experimental studies described in the previous chapter were designed to develop alternative formulations to avoid the problems of plugging. Conclusions reached on the basis of the experimental studies can be used to identify criteria for selecting nutrient formulations for a particular site.

1. Concentration of Phosphate

The required concentration of orthophosphate for microbial use under oxygen-limiting conditions, such as those expected in bioremediation operations, is not well known. The following criteria may be used for choosing a phosphate concentration for field application:

a. Available Phosphorous Content. According to the tests performed in this study, an available (or exchangeable) phosphate content of ~20 mg/L in the soil may be sufficient to provide excess phosphorous for microbial growth.

b. Type of Soil. If phosphate is to be added, either because the P-content of the soil is low or based on a conservative estimate of microbial requirements, the soil characteristics should be considered. Calcareous soils, such as from the Homestead AFB, Florida, have a high Ca content and a high sorption capacity. In such soils, addition of phosphate should be avoided. Nearly all added phosphate in calcareous soils will probably be adsorbed or precipitated.

In sandy soils, such as from the Eglin AFB, phosphate may be added in nutrient formulations. A combination of orthophosphate and TMP may be used to minimize precipitation. Phosphate levels up to ~20 mg/L may be achieved by adding phosphate in the form of orthophosphate. For higher concentrations, phosphate should be added as TMP or a mixture of TMP and orthophosphate. The

concentrations of TMP and orthophosphate may be estimated based on column experiments similar to those described in this study.

2. Micronutrients

Common micronutrients for microbial growth include Cu, Zn, Mo, MN, B, Fe, and S. The micronutrient concentrations in nutrient formulations may be based upon the groundwater composition. Trace amounts of most the above elements generally are present in groundwaters (White et al., 1963). Thus, a complete analysis of the local groundwater should be obtained before choosing the micronutrients for a particular site.

3. Phosphate Precipitation in the Aquifer

Adding the nutrient solutions will disturb the geochemical conditions in the aquifer. If phosphate is being used in the nutrient formulations, Ca and Fe phosphate phases also are likely to precipitate.

The impact of phosphate mineral precipitation at a site may be evaluated on the basis of long-term bench-scale tests. Short-term tests are likely to be deceptive and may suggest a much lower precipitation potential than what may occur in situ. A long-term test may be designed as follows.

Soil from the contaminated zone should be eluted in a column with nutrient-spiked, site-specific groundwater in a recirculatory flow path. Alternatively, a batch reactor should be assembled with a soil/solution ratio of less than two. The batch reactor should be placed on a mechanical shaker for maximum reaction. The concentrations of Ca, Fe and P in the system should be measured over a period of at least 30 days. The volume of precipitated minerals per unit volume of nutrient solution may then be calculated by the following equations, assuming that equilibrium has been reached:

$$V_{\text{total-P}} \ (\text{cm})^3 = V_{\text{Ca-phos.}} + V_{\text{Fe-phos.}} \qquad \text{(E-12)}$$

$$V_{\text{Ca-phos.}} = \frac{M_{Ca}}{8} \times 0.16 \qquad \text{when } M_{Ca} < \frac{3}{5} M_{PO4} \qquad \text{(E-13)}$$

or

$$= \frac{M_{PO4}}{31.7} \times 0.16 \qquad \text{when } M_{Ca} > \frac{3}{5} M_{PO4} \qquad \text{(E-14)}$$

$$V_{Fe\text{-}Phos.} = \frac{M_{Fe}}{57} \times 0.065 \qquad \text{when } M_{Fe} < M_{PO_4} \qquad \text{(E-15)}$$

Where M_i is the decrease in total concentration (mg/L) of the subscripted species during the duration of the test. The total volume of phosphates precipitated during the entire operation then is:

$$V_{total\text{-}P} \times L$$

where L is the total quantity of nutrient solutions (in liters) that are to be injected during the operation.

4. Nonbiological Oxygen Demand and Hydroxide Precipitation

Reducing conditions in the contaminated zone prior to treatment tend to increase the dissolved Fe and Mn content in the groundwater. The nonbiological oxygen demand in the aquifer is exerted predominantly by the oxidation of Fe and, to a minor extent, Mn. Thus, the Fe and Mn contents of the water used for mixing nutrient solutions should be lowered by aeration prior to re-injection.

Although results based on the Eglin AFB soil suggest that nonbiological oxygen demand may be insignificant, site-specific nonbiological oxygen demand may be estimated by conducting short-term tests similar to those described in this study. Autoclaved contaminated soil should be reacted with oxygen- and nutrient-spiked groundwater in a column. The column should be eluted with at least two pore-volumes of the solutions and then sealed for batch reaction. Dissolved oxygen concentration should be measured after reaction times of 1, 2, and 24 hours. A 1- or 2-hour experiment should be conducted after the 24-hour experiment. All experiments should be conducted consecutively after replenishing the nutrient solutions at the start of each experiment so that the nonbiological oxygen demand is not regenerated during a period of reduced oxygen concentration.

The extent of precipitation as a result of oxidizing conditions in the aquifer may be estimated by using the following relationship:

$$V_{Hydrox.}\ (cm)^3 = \frac{M_{ox}}{8} \times 0.002 \qquad \text{(E-16)}$$

where M_{ox} is the oxygen concentration (mg/L) that is estimated to be consumed by nonbiological reactions per kilogram of soil.

4. Hydrogen Peroxide Stability

The site-specific stability of hydrogen peroxide should be evaluated using a column experiment similar to that described above for the oxygen demand. Contaminated soil should be eluted with nutrient-spiked solutions containing a peroxide-stabilizing additive. This pretreatment should be carried out for approximately 60 minutes. Hydrogen peroxide should then be added to the nutrient solutions and the columns eluted for another 60 minutes. The columns should be sealed and allowed to react for 60 to 90 minutes. The stability of hydrogen peroxide should be evaluated by measuring the dissolved hydrogen peroxide concentration in the solution remaining in the column.

SECTION VI
CONCLUSIONS AND RECOMMENDATIONS

Existing nutrient formulations used to enhance in situ biodegradation of fuel hydrocarbons are less desirable for most sites because they result in (1) plugging of the aquifer due to excessive precipitation of phosphates and (2) insufficient delivery of oxygen due to rapid decomposition of hydrogen peroxide. Experimental data obtained in this study suggest that precipitation in the aquifer may be lowered by substituting polyphosphate for orthophosphate in the existing formulation. Trimetaphosphate (TMP) may be the polyphosphate of choice for sandy soils because of its slower rate of hydrolysis and its negligible adsorption on the soil.

Nutrient formulations containing any of the phosphates may not be used for biodegradation in calcareous soils, such as from the Homestead AFB, Florida. In such soils, the available Ca content and the sorption capacity of the soils are high. These properties lead to a nearly complete removal of added phosphate by sorption (adsorption and precipitation).

Screening of the inorganic and organic peroxide stabilizers identified in the literature search suggest that TMP and citrate were effective in decreasing peroxide decomposition by inorganic catalysts. Citrate indicated some potential for suppressing the enzymatic catalysts. In larger scale experiments using a 6-foot long aquifer simulator, however, the rate of peroxide decomposition was not reduced even to allow transport over a soil zone of 1.5 inches corresponding to a travel time of ~2.5 hours.

Based on the results of the present study, it is recommended that the following points should be considered while selecting nutrient formulations:

1. Determine limiting factor(s) and formulate to supply adequate, but not necessarily excess, nutrients. Do not supply unneeded chemicals.

2. Determine the solubility limitation of any nutrient salts to be added in groundwater. Do not add more nutrients than can be solubilized and transported into the aquifer.

3. If H_2O_2 is utilized as an oxygen source, determine the site-specific H_2O_2 stability and the real effectiveness of stabilizers utilized. Do not add stabilizers that are not effective or insufficiently soluble to be transported in groundwater.

4. Assess nonbiological oxygen demand. This oxygen demand must be considered when determining oxygen requirements.

5. The oxygen demand of any nutrient or hydrogen peroxide stabilizer must be taken into account. This is particularly true of ammonium as a nitrogen source, or citrate used as an iron chelator or hydrogen peroxide stabilizer.

6. The effects of increasing the redox potential of injection waters must be considered. For example, if groundwater containing ferrous iron is reinjected, the ferrous iron will both exert an oxygen demand and form a precipitate potentially causing plugging problems.

7. The toxicity of any additive must be considered, both toxicity to microorganisms responsible for biodegradation and the potential for introducing problematic contaminants (such as nitrate) to groundwater.

Although the above suggestions may help in reducing the problem of plugging in the aquifer, a major handicap to exploiting fully the in situ bioreclamation technology in the saturated zone is the lack of a suitable oxygen source. As indicated in this report, hydrogen peroxide may not be stabilized sufficiently in the soils using any of the reported catalase inhibitors. It is recommended that future research in this area include efforts (1) to evaluate the feasibility of using nitrate, pure oxygen, or other sources of oxygen for in situ biodegradation and (2) to identify an additive that may selectively inhibit peroxide-decomposing catalysts. The increased stability of peroxide, however, should not result in peroxide being toxic to the indigenous microbial population. In addition, a numerical model should be developed for predicting the performance of nutrient solutions in the aquifer. The model should simultaneously consider the transport and microbial consumption of several nutrients while any one of them may be limiting microbial growth. In addition, if species such as polyphosphates are used, the model should also address the hydrolysis of nutrients.

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APPENDIX A

TEST PLAN FOR BENCH-SCALE EXPERIMENTS

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A. INTRODUCTION

Geochemical modeling of soil-nutrient solution interactions and a review of the mechanism of hydrogen peroxide decomposition leads to the following two conclusions (see the accompanying Phase II status report): (1) existing nutrient formulations most likely would result in excessive precipitation of phosphate minerals and consequent permeability reductions during enhanced biodegradation operations; and (2) enzymatic catalysts, principally catalase, probably are the most dominant catalysts in the decomposition of hydrogen peroxide; their activity is probably not significantly decreased by the existing formulations. Based on these conclusions and a review of pertinent literature, it is recommended that polyphosphates together with several catalase-inhibitors be investigated for possible use in the nutrient formulations.

The Phase III test plan consists of 3 different groups of experiments. Their objectives are: (1) to evaluate the performance of polyphosphate compounds as alternative sources of phosphorus; (2) to evaluate the performance of possible hydrogen peroxide stabilizers that have not yet received widespread use in nutrient formulations; and (3) to develop and validate a diagnostic model for predicting the extent of precipitation at bioremediation sites. Details on the nature of the experiments and experimental design are provided below.

B. PERFORMANCE OF POLYPHOSPHATES

Phosphorus in the orthophosphate form is an essential nutrient for microbial growth. Nutrient formulations typically contain excess amounts of orthophosphate to avoid phosphorus-limited microbial growth and to increase the stability of peroxide. However, excess orthophosphate in the solutions leads to precipitation of minerals and plugging of pore spaces.

Polyphosphates are an alternative source of nutrient phosphorus because they hydrolyze in aqueous solutions to produce orthophosphate. The kinetics

of the hydrolysis reactions are dependent upon temperature, pH, microbial activity, and the concentration of the various phosphatase enzymes. Polyphosphates also appear to possess some peroxide-stabilizing properties. Hence, they may be an excellent substitute for orthophosphate in nutrient solutions.

1. Experimental Design and Methods

Polyphosphate hydrolysis experiments will be conducted in both batch systems and vertical columns. Most of the necessary kinetic data should be readily obtainable by sampling batch systems over a period of time. Some soil column studies are planned to verify the reaction kinetics from the batch studies and to obtain data on a flowing system more closely resembling the subsurface environment. The column experiments will utilize a recycling system at a known flow rate (scaled to a field application rate of 2.5 to 5 gpm) using peristaltic pumps. At least 2 different polyphosphate species, such as pyrophosphate (P_2O_7) and tripolyphosphate (P_3O_{10}) will be tested. Sampling ports will be included to permit periodic sampling of eluant and to release and trap excess carbon dioxide that may be produced by microbial respiration.

The concentrations of orthophosphate and polyphosphate in solutions from both the batch and column studies will be measured periodically. CO_2 production and O_2 depletion will be semi-quantitatively measured as an additional indicator of microbial activity. Upon completion of the column experiments, soil samples from three locations along the length of the column will be analyzed for phosphate precipitates using electron microscopy.

Chemical analyses of solutions will be performed using EPA-approved standard methodologies. Orthophosphate concentration can be measured generally by using standard wet analysis kits (such as LaMotte Chemical Colorimetric kit). However, when polyphosphates also are present in the solution, a more elaborate technique is required to measure both the ortho- and polyphosphate contents (Gilliam and Sample, 1968). The orthophosphate content is measured first using the vanadomolybdophosphoric acid (VMP) method. This method is specific for orthophosphate and does not detect any of the polyphosphates. Polyphosphates in the VMP solution are then hydrolyzed by placing the solution on a steam bath for one hour. The difference between the

analyses before and after hydrolysis is the concentration of phosphorus bound in polyphosphates.

The performance of the polyphosphates as a nutrient source will be measured by the amount of orthophosphate produced and by an increase in microbial growth. The nutrient solutions will be nutrient-spiked groundwaters from the Eglin and Homestead sites, which represent sandy and limestone lithologies, respectively. Nutrient formulations will include an ammonium and potassium salt, in addition to polyphosphate. Soils will also be from the Eglin and Homestead sites.

2. Polyphosphate Hydrolysis in Abiotic Systems

The role of solution composition in polyphosphate hydrolysis will be studied by conducting batch experiments with sterilized soils. Soils and groundwaters from Eglin and Homestead will be sterilized with mercuric chloride, as suggested by Wolf et al. (1989). Groundwaters from Eglin and Homestead will be spiked with nutrients and polyphosphates. Sterilized solutions will also be analyzed for free catalase, which may not be deactivated by the mercuric chloride.

The matrix of solution compositions will include variations in pH (5.0, 7.0, 9.0), the nature of dominant anion (bicarbonate or sulfate), and the concentration of total dissolved solids (TDS). Site-specific groundwaters will be modified to achieve these compositional differences. The results of these experiments will be compared to that of a control, containing only sterilized soil and distilled water. This control experiment will also monitor the concentrations of components leached from the soil. Overall this group of experiments is intended to isolate and evaluate the nonmicrobial component of polyphosphate hydrolysis in soils and groundwaters of varying chemistry.

3. Polyphosphate Hydrolysis in Natural, Uncontaminated Systems

Uncontaminated soils collected from the Eglin and Homestead sites will be used with nutrient-spiked groundwaters containing different concentrations of polyphosphates of varying chain lengths (pyrophosphate, tripolyphosphate, etc.). Nutrient solutions will be prepared using groundwaters from Eglin and Homestead. Soil and solution in varying soil:solution ratios will be placed in batch reaction vessels which will be continuously shaken to avoid sedimentation. Chemical analysis of both the

soil and the nutrient solutions will be performed at the beginning of the experiment. Samples will be taken periodically and then filtered and analyzed for dissolved orthophosphate content.

These experiments are intended to evaluate the microbial component of polyphosphate hydrolysis and to characterize the polyphosphate decomposition rates due to microbial activity. A thorough analysis of the effects of microbial species and all of the environmental factors that could affect the decomposition rates is beyond the scope of this study. However, the use of the site-specific soils and groundwaters from Eglin and Homestead should provide diverse microbial communities typical of many shallow soils; and the experiments will be conducted at two different temperatures to examine the effect of this potentially important variable. The results of these experiments should also lead to a better understanding of microbial degradation rates for several different polyphosphates and their optimal starting concentrations in nutrient solutions.

4. Polyphosphate Hydrolysis in Natural, Contaminated Systems

This experiment is intended to verify the results of the previous experiments using fuel-contaminated soils and flow-through columns. Data on optimal polyphosphate starting concentrations and degradation rates will be verified in fuel-contaminated soils from Eglin and Homestead at two different temperatures, if this variable was identified as important in the previous experiments. Recycling flow-through columns will be used to permit: (a) a more thorough evaluation of kinetics; and (b) an analysis of the spatial variation of any phosphate precipitates that may form in the columns. As above, orthophosphate concentrations will be monitored by withdrawing fluid periodically from in-line sampling ports.

The results of all of the experiments described in this section are intended to indicate whether polyphosphate is a viable alternative to orthophosphate as a phosphorus source in nutrient solutions.

C. PEROXIDE STABILIZATION

The current consensus appears to be that oxygen is the limiting nutrient for microbial growth during bioreclamation. When hydrogen peroxide is used as a source of oxygen, it is necessary that peroxide be transported in the contaminated zone significantly beyond the point of injection. Substantial transport of peroxide will provide elevated levels of oxygen even at the most

distant point of the contaminated zone and, therefore, maximum biodegradation of contaminants. Existing nutrient formulations use orthophosphate, and, in one case identified, minor amounts of polyphosphate to stabilize hydrogen peroxide. However, as indicated in the Phase II status report, the effect of these stabilizers is only minimal.

The literature describes several compounds that have an inhibitory effect on the activity of catalase, the most important catalyst in peroxide decomposition. The inhibitory action of these compounds has been studied only in simple biological systems, and their effectiveness in soil systems needs to be evaluated. While evaluating the extent of peroxide stabilization, it will be necessary to verify that microbial viability is being maintained to ensure that catalase inhibition is not accompanied by increased toxicity to microbes.

1. Experimental Design and Approach

Experiments to evaluate the performance of peroxide stabilizers (or catalase inhibitors) will be conducted in a horizontal flow, aquifer simulator. This simulator will be designed with a slight modification of the design of Moore et al. (1989). The simulator is made from a glass tank (-30 gallons) which contains a soil zone -15 cm high. Infiltration/production galleries are simulated by placing gravel on two sides of the soil zone. Both the soil and gravel are saturated to a height of -12 inches. The tank is sealed from the top using a glass cover. We plan to construct several simulators of varying lengths, up to -6 feet, to achieve different groundwater residence times.

Flow of solution from injection galleries through soil to production galleries is regulated by peristaltic pumps. Solutions are accessible through two ports for continuous sampling during operation. Two ports on the top of the simulator are available for sampling the gas phase.

Two baffles are created in the gravel zones to avoid channeling. The baffle in the infiltration zone serves also to create an airtight compartment above the injection gallery. This design is particularly useful for conducting hydrogen peroxide stability experiments because the initial, rapid decomposition of peroxide at the point of injection can be monitored by measuring oxygen evolution in the airtight compartment.

In a typical peroxide stability experiment, nutrient solutions (consisting of nutrient- and peroxide-spiked groundwaters) will be applied

through the injection gallery at a rate scaled to a field application rate of 2.5 to 5 gpm. The concentrations of O_2 in the two gas ports, G1 and G2 will be monitored continuously. High oxygen concentrations in G1 will indicate a rapid decomposition of peroxide at the point of injection, while oxygen accumulation in samples from G2 will indicate some transport of peroxide. Dissolved oxygen and hydrogen peroxide concentrations will be measured in solution samples from ports S1 and S2 to evaluate the extent of peroxide transport.

Concentrations of CO_2 will be monitored in gas port G2 as an indicator of microbial growth. Solution samples from S1 and S2 will be analyzed for the concentrations of dissolved iron, calcium, phosphate (ortho and poly), nitrate, and added stabilizers (see below) to monitor precipitation and other chemical reactions.

Natural (both uncontaminated and contaminated) soils from the Eglin and Homestead sites will be used in all of the peroxide stability experiments described below. Nutrient formulations will be based on the results of the polyphosphate hydrolysis experiments conducted previously. The stabilizing effects of several different inhibitors, such as borate, fluoride, and various organic inhibitors such as citrate, will be evaluated. The first group of experiments will address individual stabilizers. These will be followed by a study of combinations of stabilizers, used either simultaneously or sequentially.

2. Stabilization by Individual Anions

a. Polyphosphates

As indicated in the Phase II status report, inhibition of hydrogen peroxide decomposition by polyphosphates occurs through an inorganic as opposed to enzymatic mechanism. Thus, a major inhibitory effect is not expected, in that enzymatic decomposition is much more rapid. Still, this needs to be explored further, because it is possible that some polyphosphates do in fact possess an enzymatic inhibitory effect that has not been reported previously.

To differentiate between microbial and nonmicrobial mechanisms, inhibition will be evaluated in both sterilized as well as unsterilized soils. However, as noted above, sterilized experiments may not be devoid of free catalase that was secreted by the microbes.

b. Borate

Borate inhibits the decomposition of hydrogen peroxide by decreasing the activity of catalase and by forming an aqueous, perborate complex which decomposes over time to yield oxygen. The range of borate concentrations required to achieve significant peroxide stabilization without a negative effect on microbial activity will first be evaluated by batch experiments, similar to those conducted by Lawes (1988). In these experiments, soil is placed in a flask, and peroxide solution is added. The flask is quickly stoppered with a ground-glass, one-hole stopper and connected via latex rubber tubing to a bent glass tube extending into the bottom of an inverted, water-filled 50-ml burette, the open end of which is immersed into a water trough. The decomposition of peroxide is then monitored by measuring the oxygen gas collected in the burette.

The levels of borate estimated from the batch studies will then be added to the nutrient formulations to be used in the aquifer simulator. The sequence of borate application (i.e., before or concurrent with peroxide) will be varied to evaluate fully the effect of borate on peroxide stability.

c. Other Inhibitors

Numerous other possible inhibitors of catalase were identified in the Phase II status report. These include fluoride, sulfide, hypophosphite, and a number of organic inhibitors, including pyrogallol, hydroquinone, catechol, and resorcinol. Compounds will first be tested for significant inhibition in simple batch experiments before proceeding with the more complex simulator experiments. The goal is to identify a compound or groups of compounds that possess strong inhibitory power and selectivity for deactivating catalase. The search for additional inhibitors for experimental study, based on the literature and consultation with biochemical authorities, will be continued during the design phase of the project.

d. Stabilization by combinations of anions

The objective of this effort will be to identify synergistic effects between two or more catalase inhibitors. Combinations of anions will be tested in batch experiments, followed by simulator experiments when positive synergisms are identified.

D. DIAGNOSTIC PROCEDURES

The objective of this activity is to develop a simple model for predicting the severity of precipitation reactions that may occur at a site being considered for in situ bioreclamation. The model will also be designed to indicate what types of chemicals should be avoided in nutrient formulations to minimize plugging. This activity is divided into three steps: (a) model development, (b) model validation, and (c) model refinement, which are discussed further below.

1. Model Development

The first step will be to identify the critical variables and input parameters, which will include but not necessarily be restricted to groundwater chemistry, soil chemistry, redox buffering capacity, soil porosity, and nutrient formulation injection rates. The model will basically constitute a generalized mathematical expression of the sample calculation performed for Eglin AFB on page 3 of the Phase II Status Report for this project. The principal precipitation reactions of concern will include the formation of insoluble metal phosphates, as well as those that form as a result of oxygenation, such as ferric hydroxide.

2. Model Validation

A series of laboratory experiments will be conducted to validate and refine the numerical model developed above. The first set of experiments will be simple batch mixing experiments utilizing nutrient formulation (ranging from high to low orthophosphate concentration) and synthetic groundwater (high and low hardness and alkalinity). Mixtures will be adjusted to a range of pH values that might be encountered in the field, allowed to equilibrate, and then filtered. The amount and composition of any precipitates formed will then be evaluated. The second set of experiments will be similar to the first, except that actual groundwater from the Eglin (low hardness and alkalinity) and Homestead (high hardness and alkalinity) sites will be substituted for synthetic groundwaters. After equilibration with nutrient formulation containing variable phosphate concentrations, the amount and composition of precipitates will be determined.

The third set of experiments will utilize a nutrient formulation/groundwater/soil system and will be conducted in flow-through

columns. Soils will be either carbonate lithologies from the Homestead site or sands collected from Eglin. The presence of the soils may lead to reactions not observed in the single liquid:liquid mixing experiments, induced, for example, by changes in chemistry or enhanced nucleation due to the presence of fine soil particles. Nutrient formulation containing variable phosphate concentrations will be spiked into actual groundwater flowing through the soil column. The extent of precipitation will be evaluated based on changes in hydraulic conductivity and eluant chemistry. Also, after plugging, the soil columns will be disassembled and examined for the presence of precipitates in the pore spaces.

3. Model Refinement

The experimental results will be compared to the results predicted by the numerical model, and the model will be modified as appropriate. Any new variables or input parameters that are identified as necessary will also be added to the model at this time.

The intent of this activity is to produce a model that is simple and conservative (i.e., does not underestimate the amount of precipitation) and which can be used to predict unwanted precipitation reactions based on laboratory data collected on a site before it is too late, that is, prior to in situ bioremediation in the field. If potential problems can be diagnosed before field treatment, then many of the problems can probably be avoided by modification of the nutrient formulation or the manner in which it is added.

APPENDIX B

X-RAY DIFFRACTION ANALYSES OF SOILS

TABLE B-1. UNCONTAMINATED HOMESTEAD.

2 THETA	d	100I/IM	ID
12.005	7.37167	.91	
14.497	6.10969	.93	
16.379	5.41185	.87	
19.772	4.49022	.89	
20.942	4.24173	10.44	
21.473	4.13814	.98	
23.104	3.84953	14.68	Calcite
26.247	3.39533	15.23	Aragonite
26.627	3.34769	4.56	Quartz
27.257	3.27167	8.39	Aragonite
29.429	3.03500	100.00	Calcite
31.140	2.87200	1.03	Aragonite
31.441	2.84526	2.64	Calcite
32.762	2.73349	1.81	Aragonite
33.152	2.70219	7.62	Aragonite
36.014	2.49373	16.12	Calcite
37.295	2.41097	2.56	Aragonite
37.886	2.37474	6.31	Aragonite
38.426	2.34258	4.48	Aragonite
38.606	2.33206	3.53	Aragonite
39.437	2.28484	19.26	Calcite
41.168	2.19317	1.62	Aragonite
42.519	2.12606	2.23	Quartz
42.940	2.10622	4.94	
43.190	2.09460	12.92	Calcite
45.852	1.97900	9.66	Quartz
47.153	1.92738	8.37	Calcite
47.523	1.91323	17.31	Calcite
48.504	1.87681	20.12	Calcite
49.855	1.82907	.68	Calcite
50.225	1.81645	3.92	Quartz
51.937	1.76055	.40	Aragonite
52.457	1.74431	3.55	Aragonite
52.977	1.72839	2.31	Aragonite
56.590	1.62506	3.06	Calcite
57.421	1.60351	7.49	Calcite
58.121	1.58708	.98	Calcite
59.232	1.55994	.80	Aragonite

TABLE B-1. UNCONTAMINATED HOMESTEAD (CONCLUDED).

2 THETA	d	100I/IM	ID
60.663	1.52652	4.28	Calcite
60.934	1.52040	2.83	Calcite
61.404	1.50987	2.43	Calcite
61.814	1.50083	1.11	Aragonite
63.085	1.47362	1.90	Calcite
63.265	1.46986	1.38	Aragonite
64.667	1.44023	6.23	Calcite
65.597	1.42203	2.96	Calcite
65.797	1.41930	2.04	Aragonite
66.048	1.41453	1.24	Aragonite
66.528	1.40548	.61	
67.799	1.38111	1.88	Quartz
67.979	1.38131	1.15	Quartz
69.030	1.36052	.94	
69.210	1.35742	1.27	Calcite
69.390	1.35433	.76	
70.251	1.33984	1.34	Calcite
70.871	1.32962	.36	
72.913	1.29634	2.15	Calcite
73.093	1.29680	1.30	Calcite
73.694	1.28552	.68	Calcite
75.265	1.26254	.76	
72.296	1.24803	.92	Calcite
76.686	1.24265	.99	
77.156	1.23625	2.15	Calcite
77.917	1.22607	.67	Quartz
79.388	1.20701	.67	
80.729	1.19031	.50	Quartz
80.939	1.18775	.64	Quartz
81.530	1.17971	1.98	Calcite
81.780	1.17965	1.14	Calcite
82.260	1.17199	.88	
83.141	1.16180	.38	
83.781	1.15364	5.23	Calcite
84.032	1.15370	3.16	Calcite
84.812	1.14312	2.02	Calcite

TABLE B-2. UNCONTAMINATED EGLIN.

2 THETA	d	100I/IM	ID
20.332	4.36769	1.80	
20.552	4.32140	12.69	
20.752	4.28017	21.33	Quartz
23.935	3.71778	.34	
26.387	3.37762	32.02	
26.567	3.35512	100.00	Quartz
36.475	2.46331	9.25	Quartz
39.387	2.28763	4.65	Quartz
40.217	2.24227	2.74	Quartz
42.379	2.13277	4.30	Quartz
45.422	1.99674	.75	
45.602	1.98927	1.28	
45.732	1.98391	2.67	Quartz
49.545	1.83979	.17	
50.055	1.82222	8.14	Quartz
50.405	1.81038	.47	Quartz
54.799	1.67517	3.65	Quartz
55.219	1.66341	1.02	Quartz
57.161	1.61144	.31	
59.883	1.54333	7.57	Quartz
61.404	1.50870	.78	Quartz
61.554	1.50911	.45	Quartz
63.966	1.45431	1.17	Quartz
64.166	1.45385	.59	Quartz
65.727	1.41953	.30	Quartz
67.649	1.38381	4.57	Quartz
68.079	1.37611	6.56	Quartz
68.239	1.37327	7.43	Quartz
68.409	1.37367	3.18	Quartz
69.911	1.34447	.12	
73.413	1.28873	1.70	Quartz
73.614	1.28891	.93	Quartz
75.595	1.25687	3.67	Quartz
75.815	1.25687	1.96	Quartz
77.597	1.22937	1.04	Quartz
77.817	1.22948	.58	Quartz
79.828	1.20052	3.04	Quartz
80.049	1.20074	1.82	Quartz
81.059	1.18536	1.80	Quartz
81.410	1.18115	2.11	Quartz
81.680	1.18084	1.00	Quartz
83.761	1.15386	2.49	Quartz
84.022	1.15381	1.27	Quartz
84.892	1.14136	.22	Quartz

TABLE B-3. CONTAMINATED EGLIN.

2 THETA	d	100I/IM	ID
10.370	8.524	0	
20.830	4.261	11	Quartz
26.640	3.343	100	Quartz
29.430	3.033	0	Calcite
36.520	2.458	4	Quartz
39.480	2.281	5	Quartz
40.270	2.238	4	Quartz
42.430	2.129	4	Quartz
45.800	1.980	4	Quartz
50.140	1.818	11	Quartz
54.850	1.672	3	Quartz
55.330	1.659	2	Quartz
57.230	1.608	0	Quartz
59.940	1.542	5	Quartz
60.140	1.537	2	
64.030	1.453	1	Quartz
65.750	1.419	0	Quartz
65.980	1.415	0	
67.740	1.382	3	Quartz
68.330	1.372	7	Quartz
68.570	1.367	2	
73.440	1.288	1	Quartz
73.680	1.285	0	
75.660	1.256	1	Quartz
75.920	1.252	1	
77.660	1.229	1	Quartz
77.880	1.226	0	
79.870	1.200	2	Quartz
80.090	1.197	2	Quartz
81.180	1.184	1	Quartz
81.500	1.180	3	Quartz
81.750	1.177	1	
83.820	1.153	1	Quartz
84.100	1.150	0	
84.940	1.141	0	Quartz
85.220	1.138	0	
84.420	1.115	0	

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APPENDIX C

DESCRIPTION OF THE SOLUTE TRANSPORT MODEL

APPENDIX C

DESCRIPTION OF THE SOLUTE TRANSPORT MODEL

A. INTRODUCTION

An oxygen/phosphate transport model is presented in Figure C-1. This model is referred to in the following sections.

1. ADVECTION - DISPERSION

A one-dimensional model for solute transport in a homogeneous isotropic porous medium with a steady state uniform flow (Javandel et al. 1984) is given by

$$D \frac{\partial^2 C}{\partial x^2} - V \frac{\partial C}{\partial x} = R \frac{\partial C}{\partial t} \qquad \text{(C-1)}$$

where C = solute concentration

x = distance, t = time

D = dispersion coefficient

v = seepage velocity

R = retardation factor, and is given as:

$$R = \left[\frac{1 + \rho_b K_d}{n}\right] \qquad \text{(C-2)}$$

where n = effective porosity

K_d = distribution coefficient

ρ_b = bulk density of the solid

The solution of the above equation can be written in the form,

$$C(x,t) = C_o \exp(-\alpha t) A(x,t) \qquad \text{(C-3)}$$

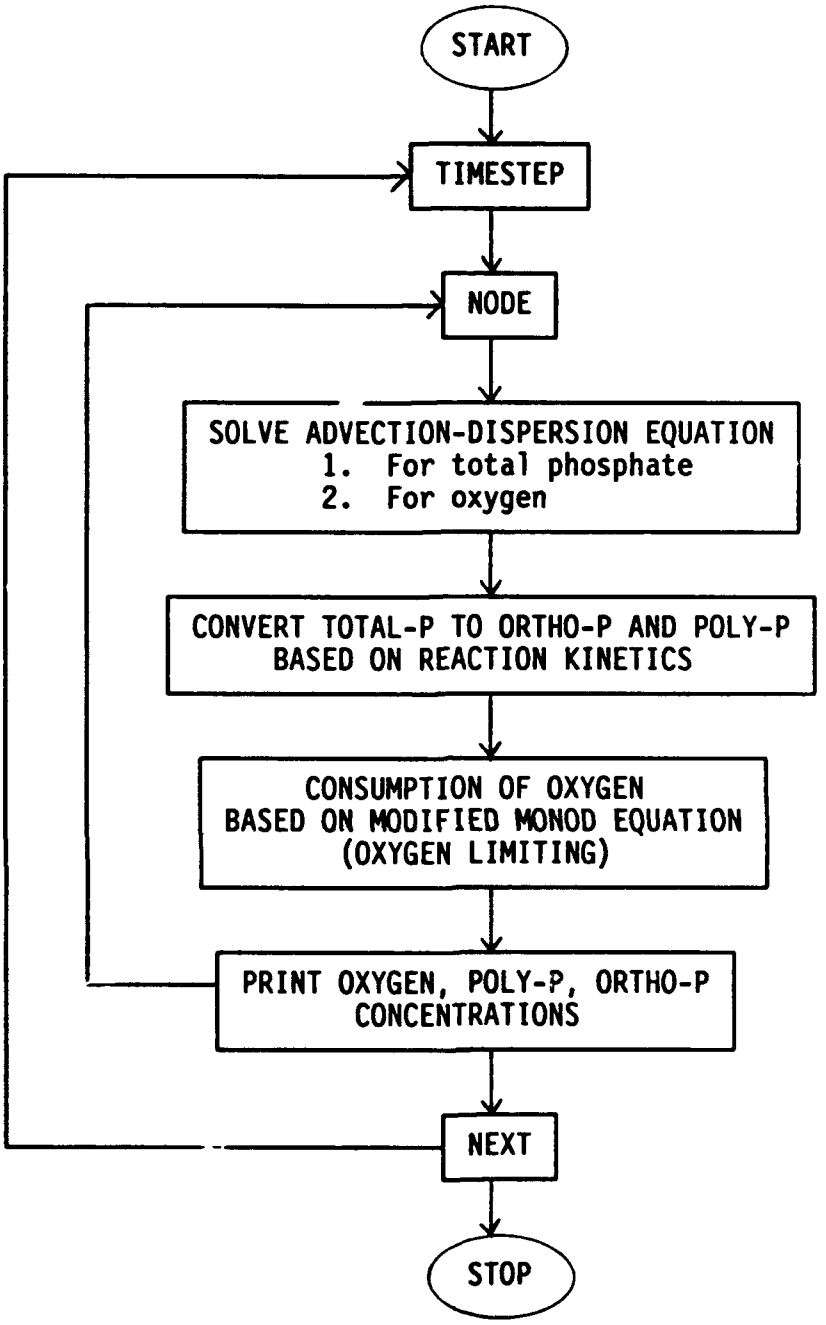

Figure C-1. Oxygen/Phosphate Transport Model.

where C_o is the concentration of a solute in the recharge fluid, and α = constant. When $\alpha = 0$, a constant input concentration of C_o is implied. The parameter values used are,

V = 1.5 m/day
D = 0.06 m^2/day
R = 1 (no retardation due to adsorption)
α = 0 (constant input concentration)
C_o = 40 mg/L for oxygen
= 280 mg/L for total phosphate.

The distances (x) of the sampling wells from the interface between the soil and the gravel zone are as follows:

well # 9 = 0.04 m (1.8 in)
8 = 0.20 m (7.9 in)
7 = 0.30 m (12.0 in)
6 = 0.40 m (16.0 in)
5 = 0.52 m (20.5 in)
4 = 0.60 m (23.8 in)
3 = 0.72 m (28.5 in)
2 = 0.82 m (32.3 in)
1 = 1.02 m (40.3 in)

The solute was transported through evenly spaced nodes, 0.02 meters apart.

2. PHOSPHATE KINETICS

The hydrolysis of polyphosphates is given by the following reactions and rate constants K_1, K_2, and K_3:

$$P_3O_9^{-3} \xrightarrow{K_3} P_3O_{10}^{-5} \qquad \text{(C-4)}$$

$$P_3O_{10}^{-5} \xrightarrow{K_1} P_2O_7^{-4} + PO_4^{-3} \qquad \text{(C-5)}$$

$$P_2O_7^{-4} \xrightarrow{K_2} 2PO_4^{-3} \qquad \text{(C-6)}$$

If the concentrations are represented by

(PPP) = concentration of $P_3O_9^{-3}$, (PPP') = concentration of $P_3O_{10}^{-5}$

(PP) = concentration of $P_2O_7^{-4}$

(P) = concentration of PO_4^{-3},

the differential equations for the hydrolysis reactions are

$$-\frac{d(PPP)}{dt} = K_3 (PPP) \qquad \text{(C-7)}$$

$$\frac{d(PPP')}{dt} = K_3 (PPP) - K_1(PPP') \qquad \text{(C-8)}$$

$$\frac{d(PP)}{dt} = K_1 (PPP^1) - K_2 (PP) \qquad \text{(C-9)}$$

These equations can be solved as first-order differential equations with the following solutions:

$$(PPP) = (PPP_o)e^{-K_3 t} \qquad \text{(C-10)}$$

$$(PPP') = (PPP_o)K_3 \left\{ \frac{E^{-K_3 t}}{(K_1-K_3)} + \frac{e^{-K_1 t}}{(K_3-K_1)} \right\} \qquad \text{(C-11)}$$

$$PP = \frac{K_1 K_3}{(K_1-K_3} (PPPo) \left[\frac{e^{-K_3 t}}{(K_2-K_3} - \frac{e^{-K_1 t}}{(K_2-K_1)} \right] + D \qquad \text{(C-12)}$$

$$D = \frac{(PPo) + \frac{K_1 K_3 (PPPo)}{(K_2-K_3)(K_2-K_1)}}{e^{-K_2 t}} e^{-K_2 t} \qquad \text{(C-13)}$$

$$(P) = PPPo \left[\frac{K_1 e^{-K_3 t} - K_3 e^{-k_1 t}}{(K_3 - K_1)} \right]$$

$$+ 2K_2 \left[\frac{(PPPo)}{(K_1 - K_3)} \left\{ \frac{K_1 e^{-K_3 t}}{(K_3 - K_2)} + \frac{K_3 e^{-K_1 t}}{(K_2 - K_1)} \right\} \right.$$

$$\left. - \frac{K_1 K_3 (PPPo) e^{-K_2 t}}{K_2 (K_2 - K_3)(K_2 - K_1)} \right] + D'$$

$$D' = (Po) + (PPPo) - 2K_2 \left[\frac{(PPPo)}{(K_1 - K_3)} \left\{ \frac{K_1}{(K_3 - K_2)} \right. \right.$$

$$\left. + \frac{K_3}{(K_2 - K_1)} \right\}$$

$$\left. - \frac{K_1 K_3 (PPPo)}{K_2 (K_2 - K_3)(K_2 - K_1)} \right] \qquad \text{(C-14)}$$

Here (Po, (PPo) and (PPPo) are initial concentrations (at t = o).
The rate constants used are

K_1 = 1.54 E-4 /S
K_2 = 3.0 E-6 /S
K_3 = 1.0 E-5 /S for contaminated zone
= 9.0 E-6 /S for uncontaminated zone.

Since the phosphates undergo transport as well as hydrolysis, the following procedure is used for tracking polyphosphates and orthophosphates.

Step 1: Transport equation:

Solve the transport equation for node 'n' to get P_n, and O_n (Total-P and oxygen concentration before kinetics and biodecay).

Step 2: At nth node:

(A) Total-P = P_n (before kinetics and biodecay)

Poly-P = $\hat{Y}_n$ (before kinetics and biodecay)

Ortho-P = $\hat{R}_n$ (before kinetics and biodecay)

Calculate ratios 'a' and 'b'

$a = \frac{Y_{n-1}}{P_{n-1}}$ = fraction of poly-P at previous node after kinetics

$\frac{R_{n-1}}{P_n\text{-}1}$ = fraction of ortho-P at previous node after kinetics

then $\hat{Y}_n = a\,\hat{P}_n$, $\hat{R}_n = b\,\hat{R}_n$

Note: Initial condition is a = 1, b = 0 at n = 0 since we begin with no ortho-P.

Call subroutine 'KINETICS'

so that $Y_n \rightarrow R_n' + Y_n$ over timestep Δt

Y_n = poly-P concentration at node 'n'

R_n' = amount of ortho-P produced from hydrolysis of $\hat{Y}_n$

$R_n'' = \hat{R}_n' + R_n'$ = ortho-P concentration after hydrolysis.

3. BIODECAY

The oxygen consumption for the limiting case is given by Molz et al. (1986).

$$\frac{\partial O}{\partial t}\left(1 + \frac{a_o A_s}{n}\right) = -\frac{D_{ob}}{n}\left[\frac{O - \Theta}{\delta}\right] N_c \pi r_c^2 \qquad \text{(C-15)}$$

$$D_{ob} \left[\frac{0 - \theta}{\delta}\right] \pi r_c^2 = \gamma \mu_m M_c \left[\frac{\theta}{K_o + \theta}\right] + \alpha K_d M_c \left[\frac{\theta}{K'_o + \theta}\right] \quad \text{(C-16)}$$

This is the modified form of the Monod equation. It assumes that the consumption of oxygen by the microbial colonies is determined by the concentration of oxygen in a microbial colony, θ. This microbial colony with mass M_c is cylindrical in shape with a radius of r_c. The colony oxygen concentration θ is determined by diffusion from the bulk fluid where the oxygen concentration is 0, across a diffusion layer of thickness δ. D_{ob} is the diffusion coefficient.

N_c = no. of colonies
n = porosity of the medium
A_s = effective specific surface of the aquifer matrix
α_o = adsorption coefficient
μ_m = maximum specific growth rate
K_o' = oxygen saturation constant for maintenance
K_d = microbial decay coefficient.

Solution of Equation (C-16) gives

$$\theta = \frac{\left[-B+C+A(K_o-\hat{0})\right] + \left[B+C+A(K_o-\hat{0})\right]^2 - 4C}{2} \quad \text{(C-17)}$$

$$C = A\hat{0}K_o \quad \text{(C-18)}$$

$$A = \frac{D_{ob}\,\pi r_c^2}{\delta}, \quad B = \gamma\,\mu m\, mc, \quad C = \alpha K_d M_c \quad \text{(C-19)}$$

For a small finite timestep Δt, Equation (A-1) becomes

$$0 = \hat{0} - \frac{D_{ob} \cdot \Delta t}{nR}\left[\frac{\hat{0} - \theta}{\delta}\right] N_c \pi r_c^2 \quad \text{(C-20)}$$

Also, from Molz et al. (1986)

$$\frac{1}{N_c}\frac{\delta N_c}{\delta t} = \mu_m \left[\frac{\theta}{K_o + \theta}\right] - K_d \quad \text{(C-21)}$$

For a finite timestep Δt, Equation (C-21) becomes

$$N_c = N_c' \left[1 + \Delta t \left\{ \mu_m \left(\frac{\Theta}{Ko+\Theta} \right) - K_d \right\} \right] \qquad \text{(C-22)}$$

N_c' = number of colonies at noden in the previous timestep.

The parameter values used are

K_d = 0.02 days $^{-1}$
D_{ob} = 0.71 cm^2/day
K = Ko' = 0.77E-3 mg/cm^3
N_c = 1.0 E-5
μ_m = 4.34 days^{-1}
M_c = 90 mg/cm^3
α = 0.0402
δ = 0.05 cm

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APPENDIX D

CONCENTRATIONS OF CA, MG, AND FE IN THE AQUIFER SIMULATOR EXPERIMENT

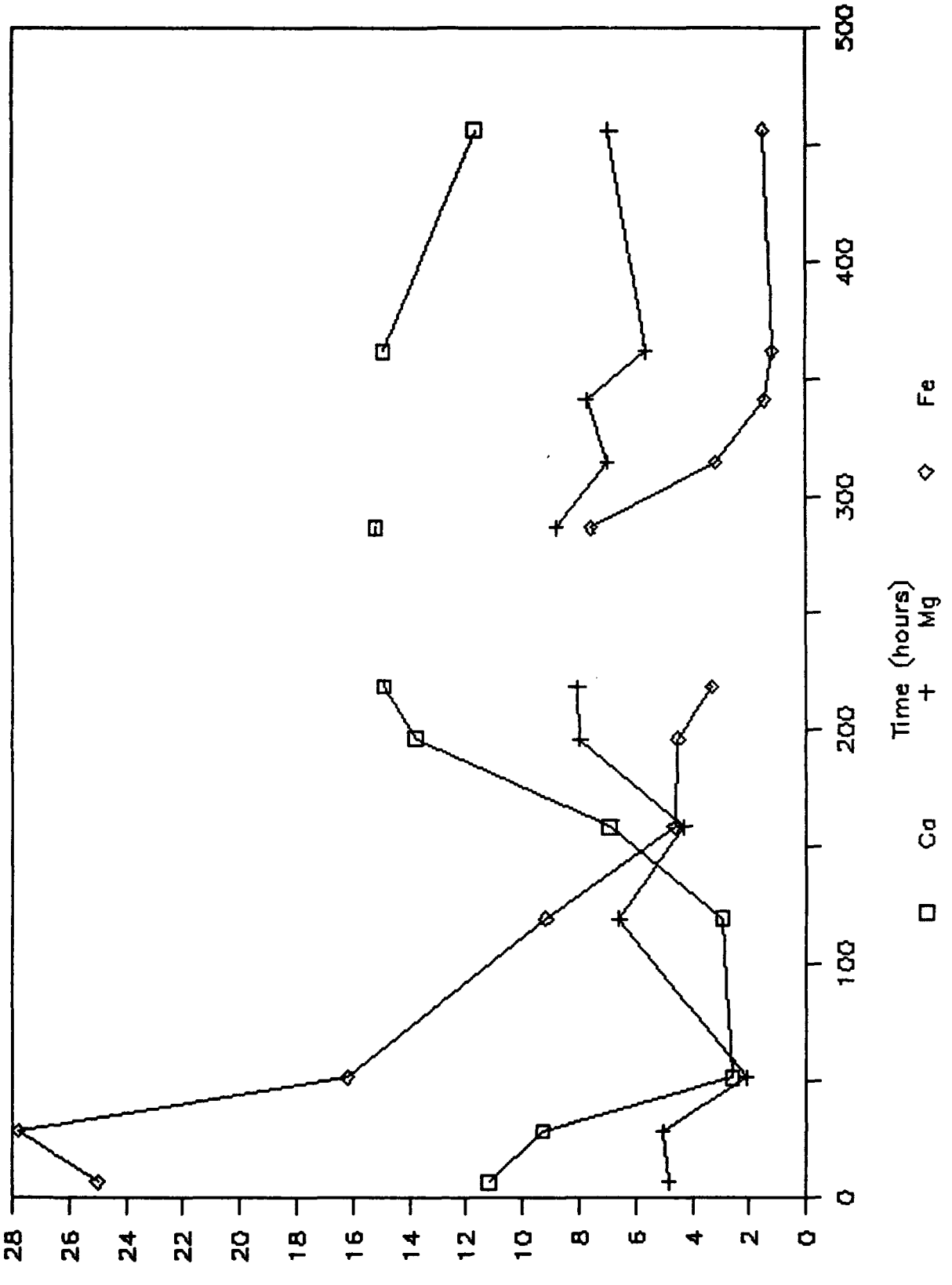

Figure D-1. Ca, Mg, and Fe Concentrations in Well 1.

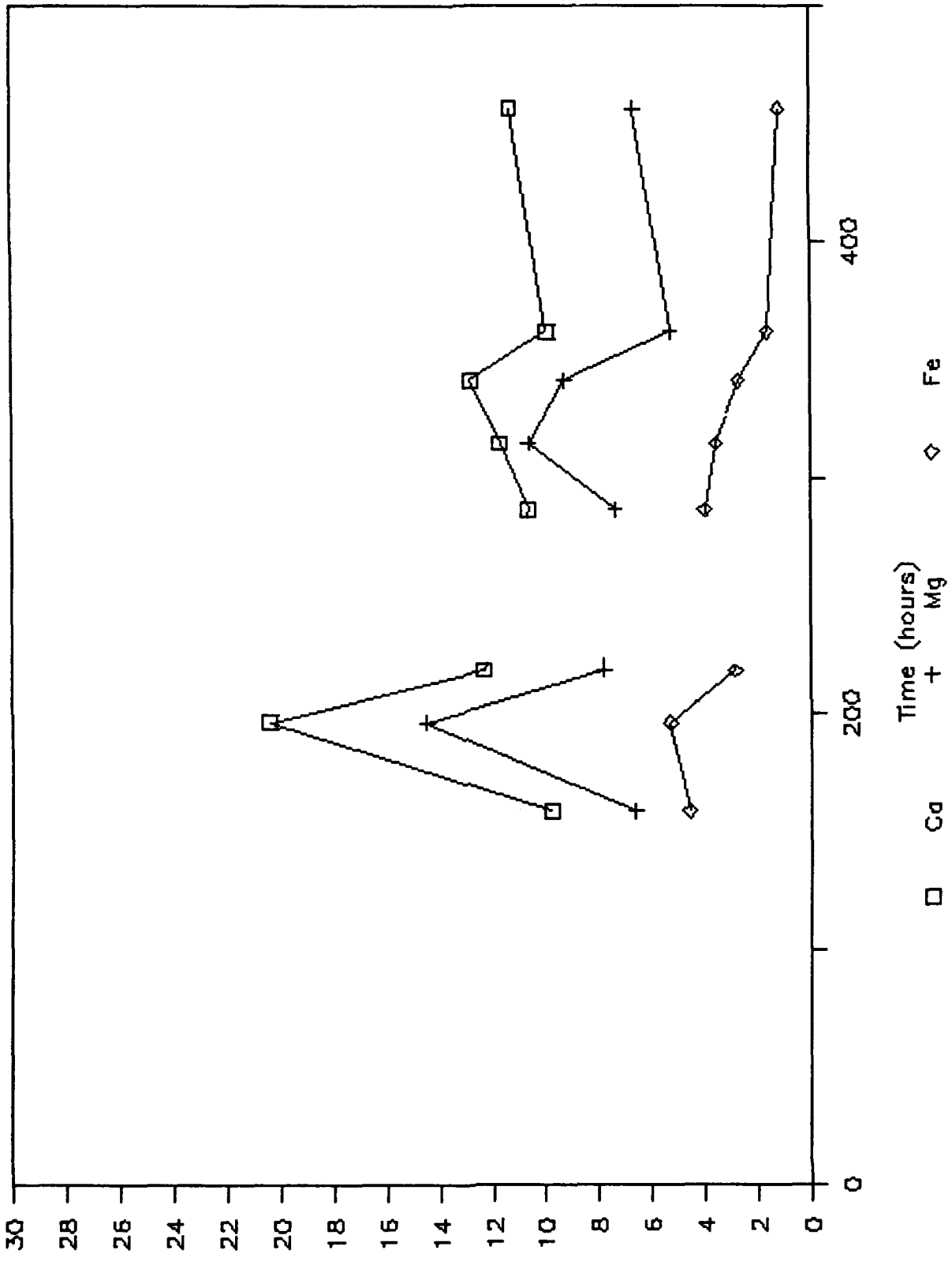

Figure D-2. Ca, Mg, and Fe Concentrations in Well 2.

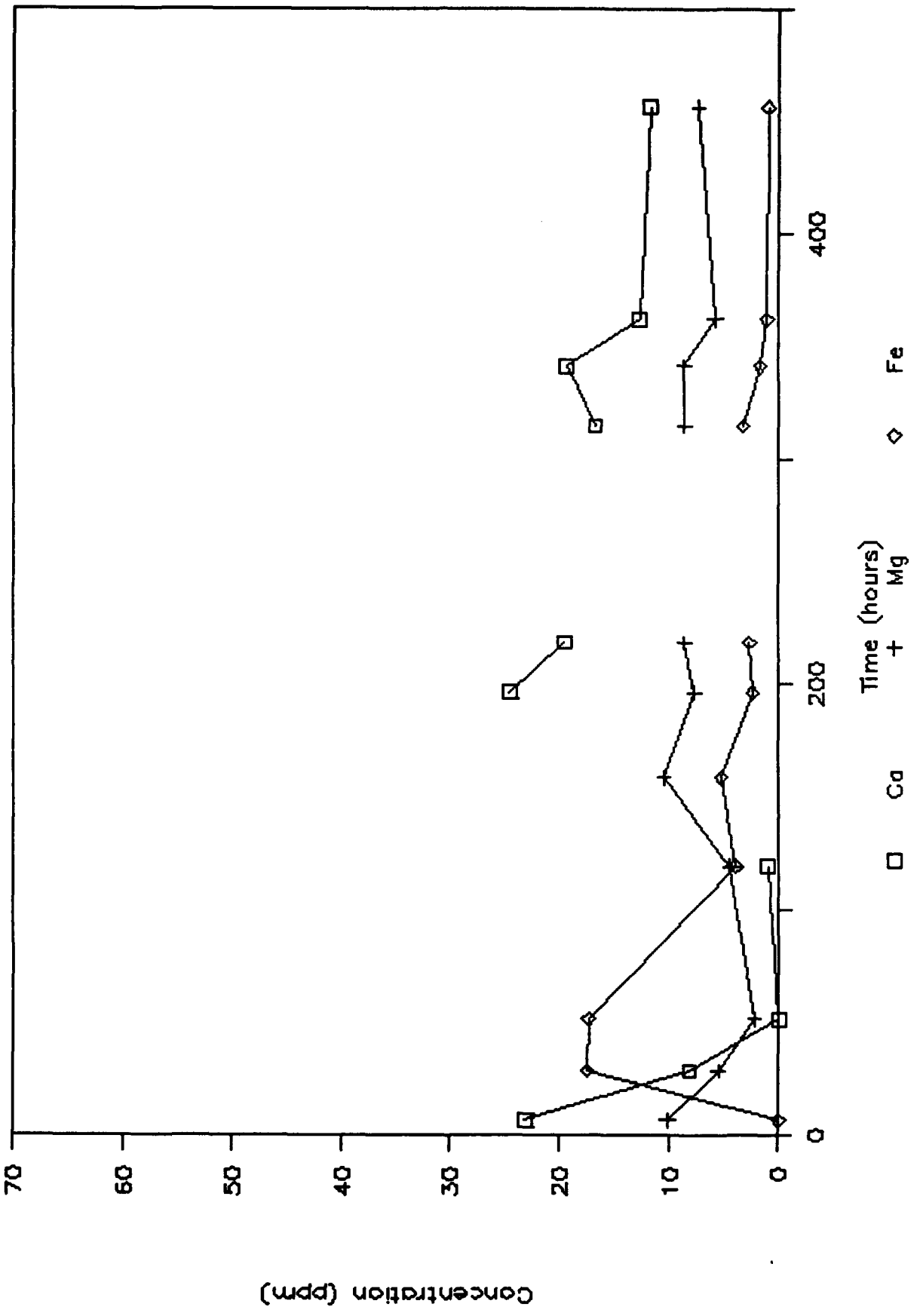

Figure D-3. Ca, Mg, and Fe Concentrations in Well 3.

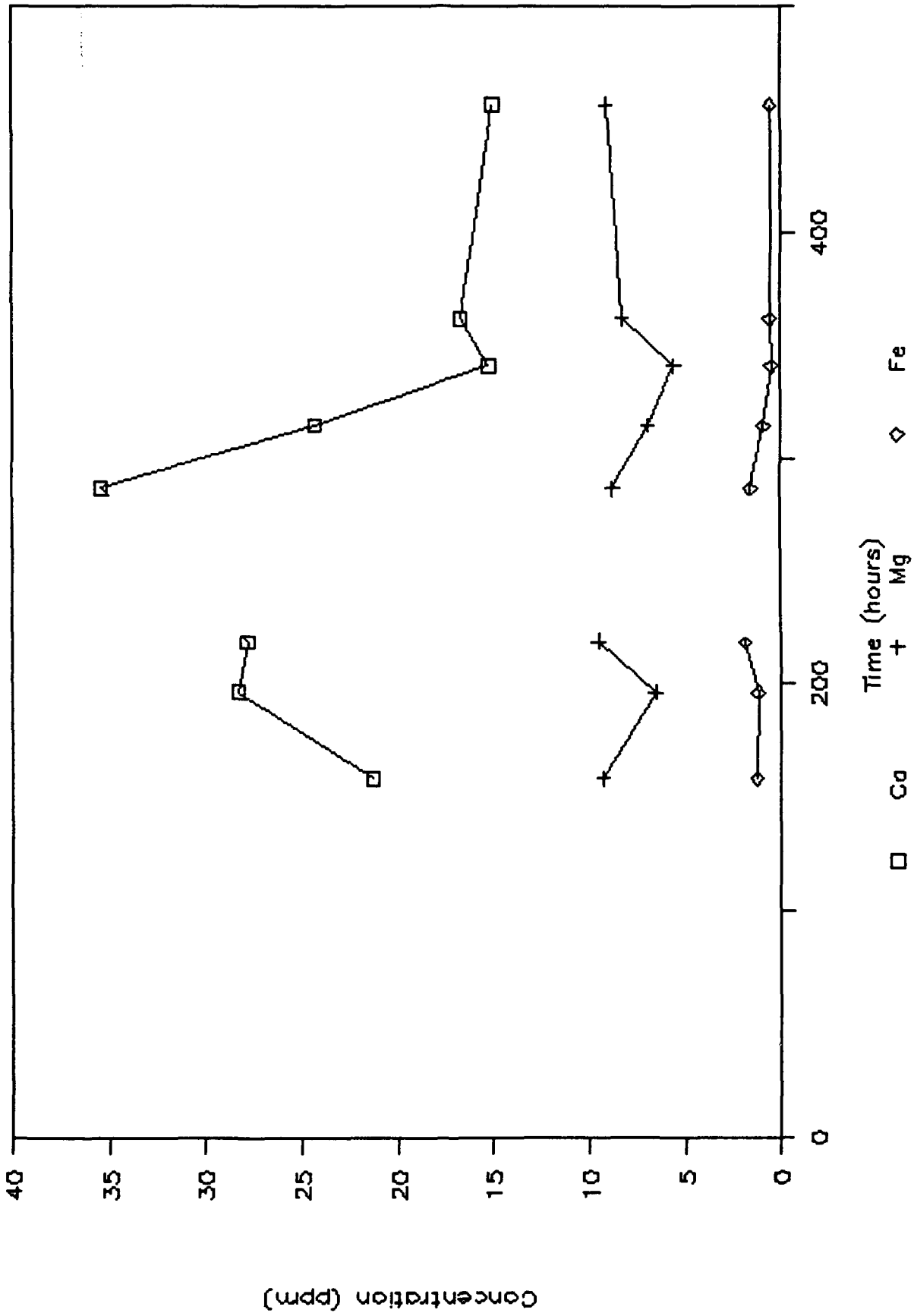

Figure D-4. Ca, Mg, and Fe Concentrations in Well 4.

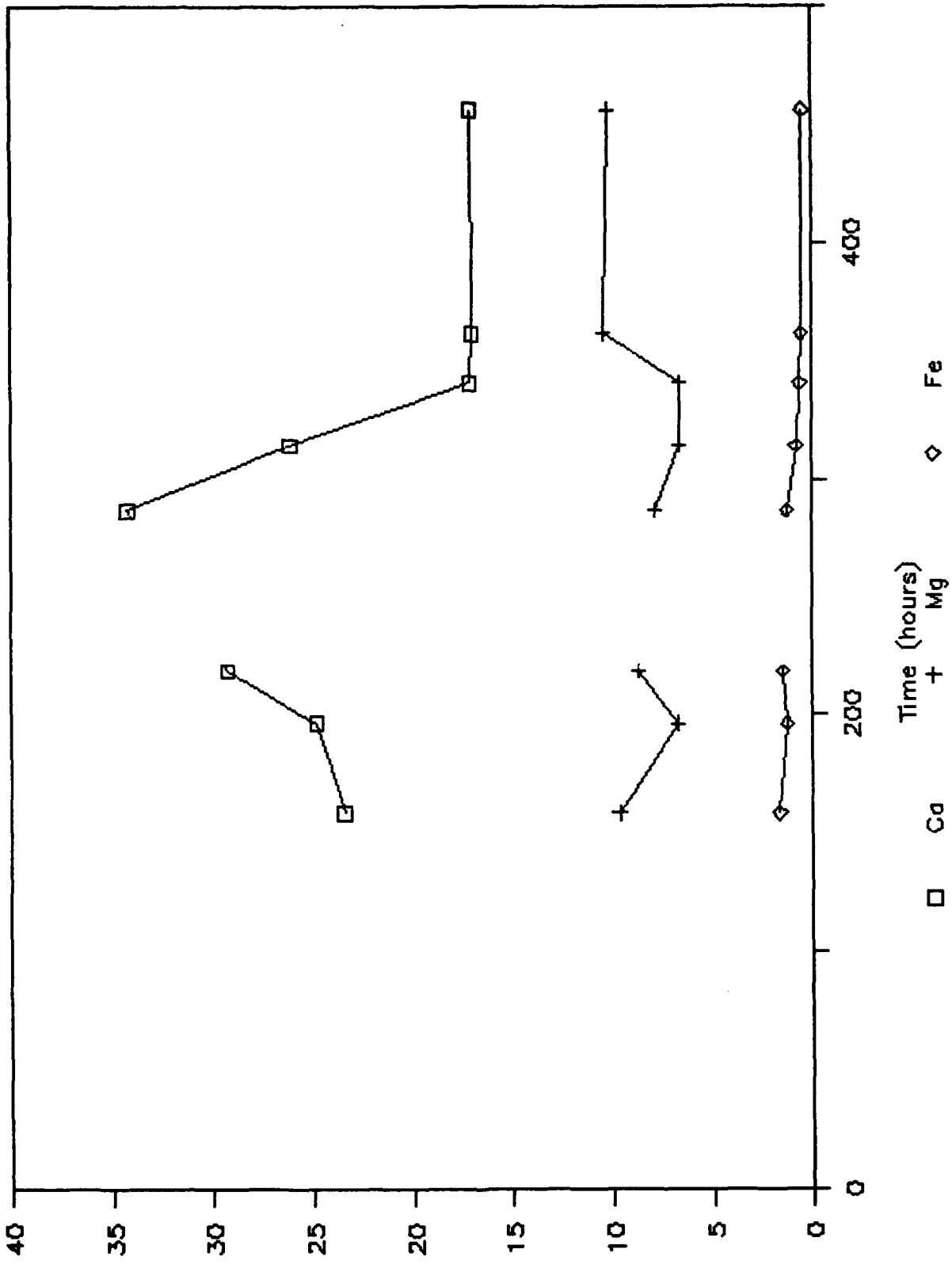

Figure D-5. Ca, Mg, and Fe Concentrations in Well 6.

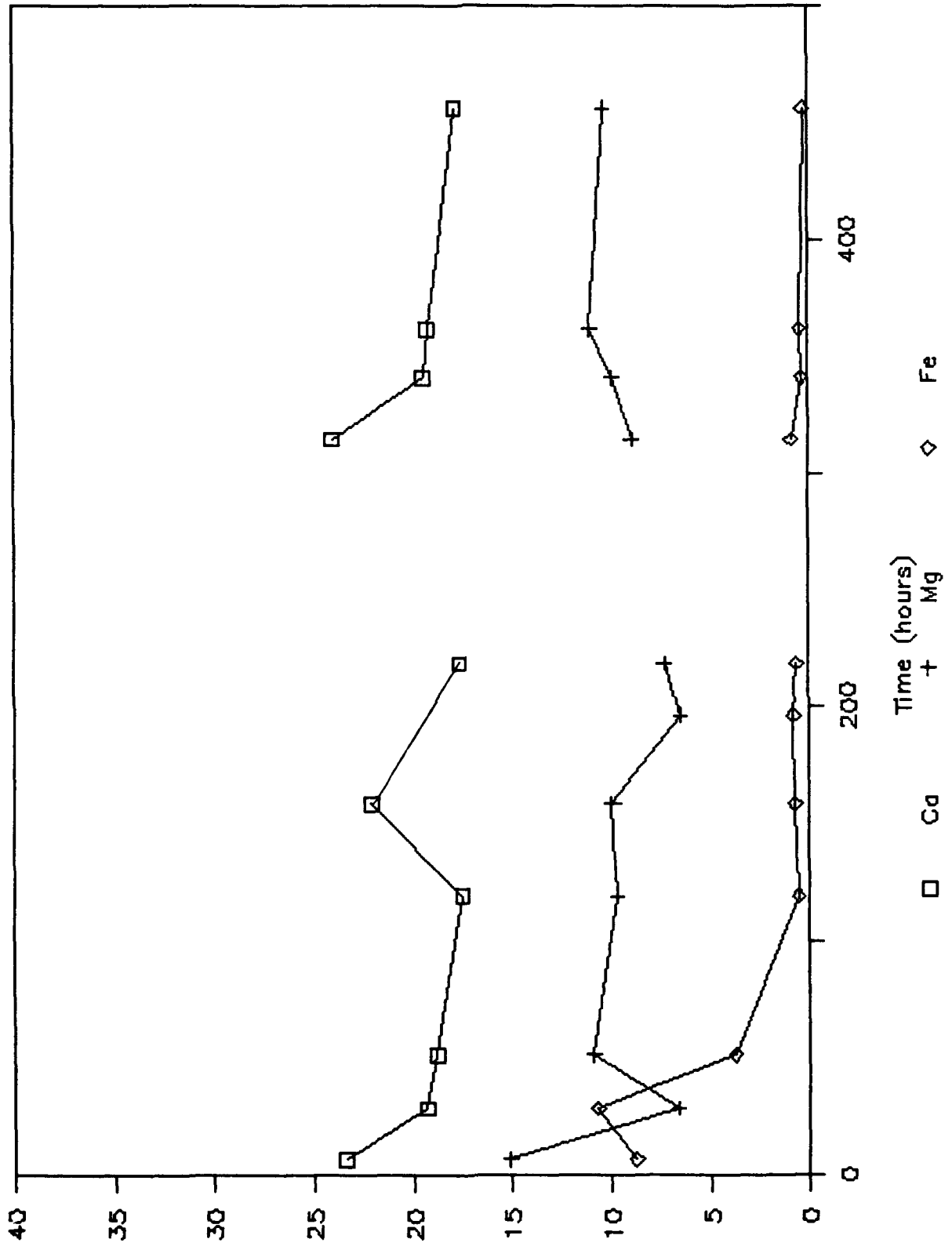

Figure D-6. Ca, Mg, and Fe Concentrations in Well 7.

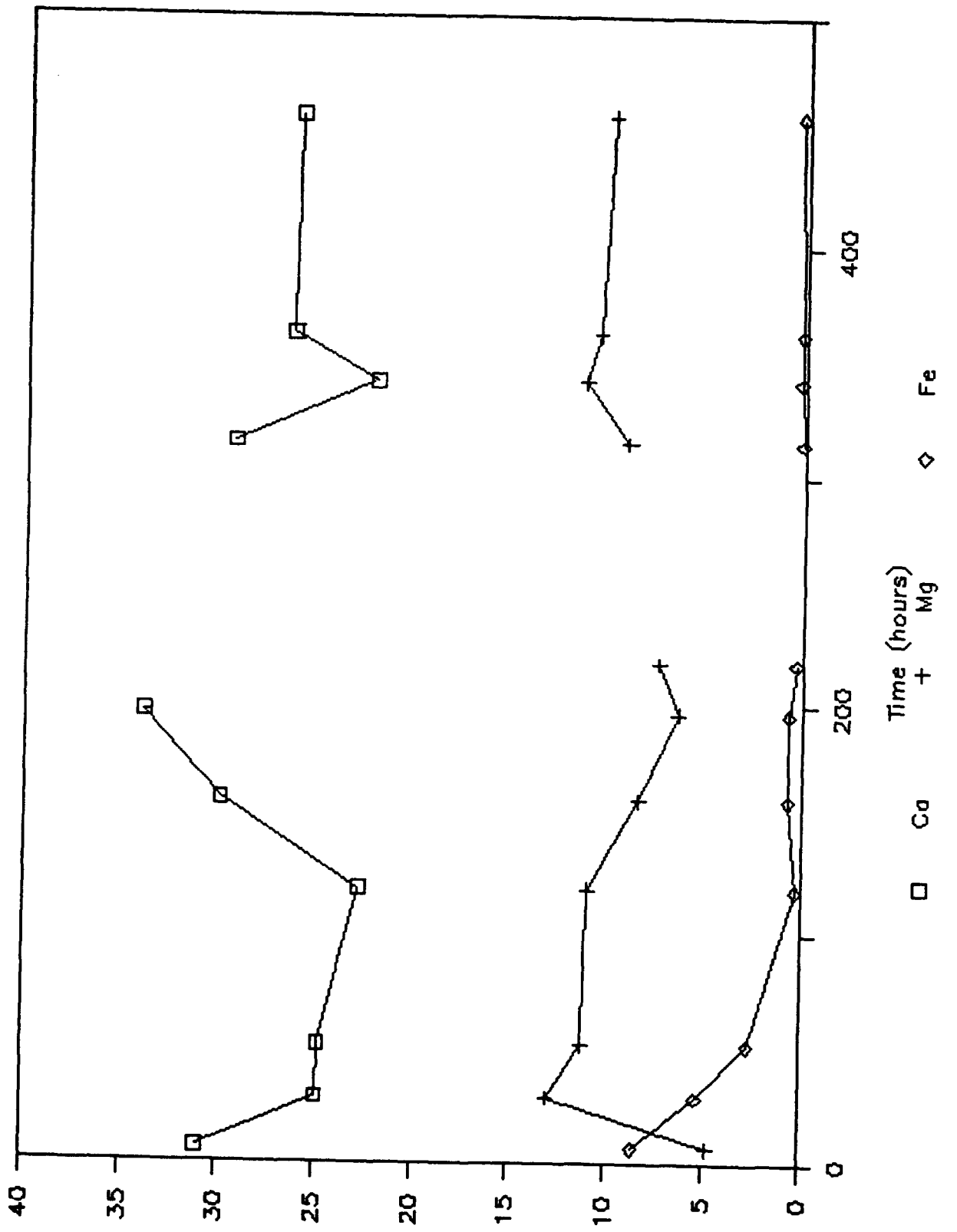

Figure D-7. Ca, Mg, and Fe Concentrations in Well 8.

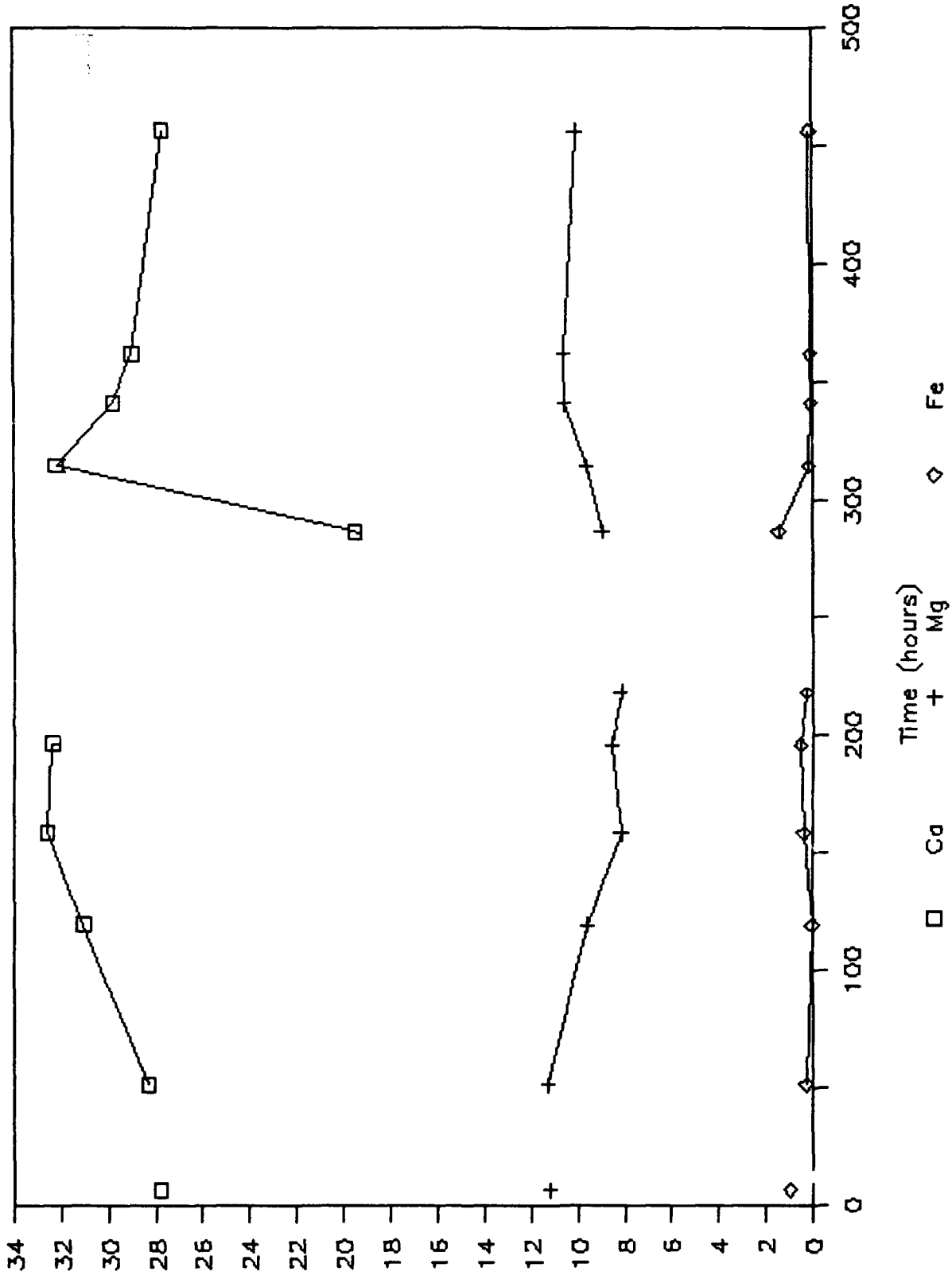

Figure D-8. Ca, Mg, and Fe Concentrations in Well 9.

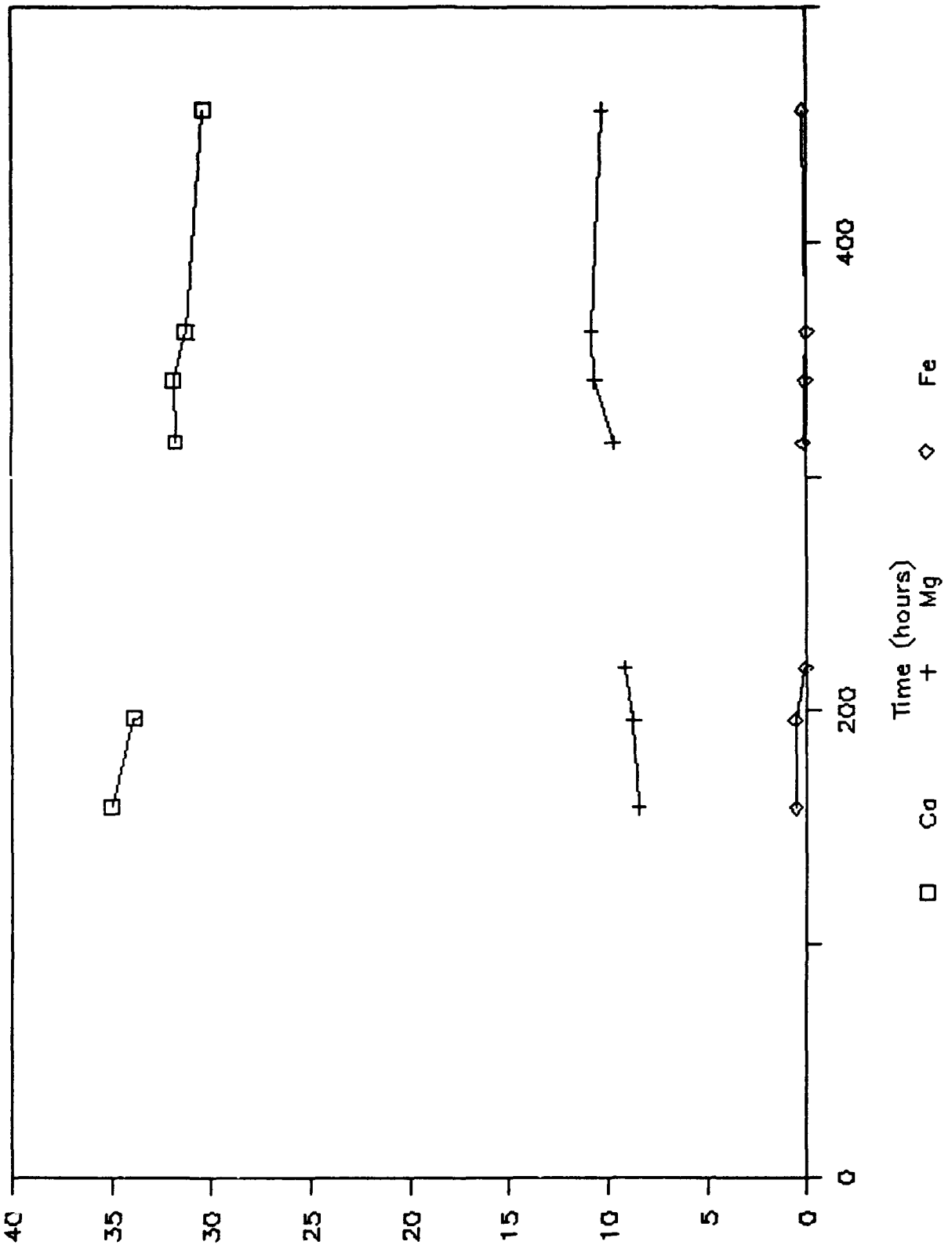

Figure D-9. Ca, Mg, and Fe Concentrations in Well 10.